THE UNIVERSITY *of* EDINBURGH

Big Cat Biogeography, Morphology and Climate Change

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Thesis for the degree of Doctor of Philosophy

The University of Edinburgh

2019

Declaration of Originality

All of the work included in this thesis is original and my own, unless indicated otherwise. The work presented in this thesis has not been submitted for any other degree or professional qualification.

Signed: _____

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28 February 2019

Abstract

This thesis investigates the long term biogeographic factors which may have contributed towards subspecific variation within modern populations of the lion (*Panthera leo*) and tiger (*Panthera tigris*). Present day morphological variation in both species is examined in light of this biogeographical modelling, existing genetic research, and further analysis of the effects of phenotypic plasticity. This research provides a spatially explicit view of the recent evolutionary history of the lion and tiger, and examines their morphology using an unprecedentedly large sample of specimens, from both captive and wild populations, and with known geographic origins. These strands of investigation are important, as it can corroborate, or challenge existing phylogenetic and morphological studies, thereby lending support to or challenging the validity of subpecific taxonomy, management units and clines. This thesis is organised around two main research themes: **1) Biogeographical range shifts in the tiger and lion:** Species distribution modelling techniques, incorporating palaeoclimatic models form the basis of understanding the changing ranges of the lion and tiger through glacial/interglacial and pluvial/interpluvial cycles of the Late Pleistocene and Holocene. Models are augmented with palaeoenvironmental evidence including fossil records, rock art and historical records and evidence of large scale stochastic events. Standard species distribution models are built upon by creating Global Environmental Zones and Strata, which aids in their descriptive power. **2) Phenotypic variation within modern populations:** Morphology is examined using an unprecedentedly large sample of linear craniometric measurements of lion and tiger specimens. The effect of phenotypic plasticity is examined by comparing the vastly different environmental conditions found between captive and wild lion and tigers. This preliminary analysis supports the interpretation of wild variation by separating the influences of life history, from evolutionary history. Geo-referenced specimens of wild lions and tigers are used to examine geographical patterns and potential environmental influences on modern day variation, in light of the biogeographical modelling and phenotypic analysis. **Core Findings:** Whilst interglacial conditions have largely benefitted the population extent of the tiger, allowing northward and westward dispersal, the opposite is true in the lion where combined interglacial and interpluvial conditions have decreased their potential range. The biogeographical modelling broadly supports contiguous potential populations of both species through changing climatic cycles, except where sea level change has isolated the Sunda populations of the tiger. Such broad scale analysis may not fully account for narrow, yet enduring barriers and conduits to dispersal such as rivers. Significant phenotypic plasticity is found within lion and tiger skull morphology, which is likely related to differences in the mechanical properties of diet. Morphological variation between wild populations is largely determined by clinal size differences in the tiger, although the similarly sized Amur and Indian populations are separable on multiple skull parameters which likely relate to phenotypic plasticity. Clinal size variation occurs less strongly in the lion with no step change in size or shape between the northern and southern subspecies. The Asian lion population shows shape differences from populations in Africa, likely due to a combination of environmental effects on skull plasticity, and the recent population bottleneck of the Asian population.

Lay Summary

This thesis investigates how long term geographic trends in populations of the lion and tiger have contributed to within species variation in modern populations. Differences in skull size and shape are put into the context of each species' distribution through time, and the effects of different life histories upon skull dimensions. Both the modelling of species past ranges, and analysis of skull morphology are important, as they can confirm or challenge existing studies of within-species variation, and therefore support or challenge the legitimacy of subspecies and conservation management units. The research themes of this thesis are split into two parts: **1) The changing ranges of the lion and tiger through long term climatic events:** Known locations of each species are associated with maps of present and past climatic data. This is used to infer the changing suitability of environmental conditions for the lion and tiger across each species' range, and through glacial and interglacial climate cycles. The models of each species' changing ranges are verified using fossil records, ancient rock art, and historical records. The models presented are built upon by determining categorical climatic classes which describe suitable and unsuitable environments for the lion. **2) Variation in skulls across modern populations:** Differences in skull size and shape between captive and wild specimens is assessed to determine how much the life of an individual animal can affect these dimensions. This analysis provides vital context for the subsequent analysis of variation of skull size and shape in the wild, by understanding the extent to which differences could be from differences in lifestyles of different populations. In combination with modelling the shifting ranges of each species, this allows a better understanding of which skull parameters are likely to have differentiated due to the different evolutionary histories of populations. **Core Findings:** Climatically, tigers have largely benefitted from interglacial conditions which have promoted forest cover in northern climates, yet the hyper-arid Saharan conditions, along with dense rainforest of the Congo basin of the present day mark a decline in the potential range of the lion since the Last Glacial Maximum. Preferred environmental conditions for both species have largely been connected during the climate cycles of the Late Pleistocene, except where interglacial sea level rise has separated tigers on the islands of Bali, Java and Sumatra from the mainland. Such a broad climatic analysis may not account well for narrow barriers and channels for dispersal such as large rivers. Lion and tiger skulls differ significantly between wild and captive specimens which is most likely due to differences in their diet. In the wild, differences between populations of the Continental tiger are dictated by gradual variation in size. Shape differentiation between the Amur population and other Continental tigers is largely determined by differences in their respective environments. Differences in skull shape between tigers on the Sunda Islands is most likely to be caused by a combination of size dependent shape changes, and the recent evolutionary separation of populations. Size changes gradually through the populations of the lion, but there are not clear shape differences between African populations. Skull shape differences between the Asian and African populations are likely caused by a combination of the effects of their different environments acting upon skull shape, and the recent population bottleneck in the Asian population.

Acknowledgements

I would like to acknowledge my supervisors, Andrew Dugmore, Andrew Kitchener, and Bruce Gittings, for their expert advice and continued support and encouragement throughout the duration of my studies and in the preparation of this thesis. I thank Nobuyuki Yamaguchi for contributing essential data, and for providing further advice on cranial morphometrics. I would also like to thank Geraldine Veron and Neil Stuart for taking the time to examine this work.

I gratefully acknowledge support from NERC PhD studentship NE/L002558/1 and the E3 Doctoral Training Partnership.

Contents

Chapter 1: Introduction

This thesis investigates Late Pleistocene and Holocene biogeographical patterns, and current morphological variation in the tiger (*Panthera tigris*) and lion (*Panthera leo*). This is important because long-term trends in population vicariance and connectivity play a pivotal role in determining micro-evolutionary differentiation; the subsequent classification of subspecies and evolutionary significant units is of great conservation importance (Karl & Bowen, 1999; Zink, 2004).

Big cats play an important role in the ecology of their respective ecosystems, and hold significant value within multiple cultures (Holland et al., 2018), yet both the lion and tiger have shown sharp and continuing population declines within the 20th and 21st centuries (Goodrich et al., 2015; Bauer et al., 2016). The currently fragmented ranges of each species represent an impoverished snapshot in time and space - robust palaeobiological baseline data are required to make crucial conservation decisions (Dietl & Flessa, 2011). Analysing the morphological variation within lions and tigers in the context of current geographical and environmental origins and long-term range shifts allows us to tease apart patterns resembling recent evolutionary differentiation from those of life-history and random variation, which feeds back into the understanding of population differentiation. In other words by understanding better the causes of patterns of morphological (and genetic) variation, the populations which are taxonomically distinct and of higher conservation importance can be determined

Examining the shifting ranges of each species over the last 2.6 million years of the Pleistocene and Holocene, collectively known as the Quaternary, sheds light upon what changes in the climate and environment have shaped their recent evolutionary histories. This biogeographical understanding complements existing genetic literature, and contextualises the analysis of morphological variation within each species.

Quaternary Environmental Change

Introduction

The Quaternary period is characterised by over two million years of global climate cycles, caused by eccentricity of the Earth's orbit, with periods of approximately 23, 41 and 100 thousand years (Imbrie & Imbrie, 1979; Imbrie et al., 1992). These cycles caused widespread interglacial and glacial periods in the northern hemisphere, with corresponding warmer and cooler global climates.

Glacial periods brought about regional changes which differ considerably from the interglacial conditions found today. The growth of ice sheets lowered global sea levels, reconnecting islands and creating new lowland habitats across continental shelves; ecological zones shifted to lower altitudes and latitudes, fragmenting some habitats and connecting others (Rohling et al., 1998; Rebelo et al., 2012; Raes et al., 2014; Binney et al., 2017). Open steppes and tundra in northern Eurasia replaced the boreal forests during interglacial periods (Binney et al., 2017). A reduction in sea levels by ~120m caused the exposure of the Sunda Shelf in South East Asia, connecting the islands of Bali, Java, Sumatra and Borneo to the mainland, and creating new terrestrial habitats characterised by marshy and humid vegetation and large rivers (Wang et al., 2009). The tropical monsoon domains, made up of a network of rainfall systems, react with similar sensitivities to changes in glacial-interglacial cycles (Chevalier et al., 2017). The rainforests of southeast Asia were considerably diminished, with the region's core characterised by savannah vegetation due to increased seasonality through changes to the monsoonal forcing (Gathorne-Hardy et al., 2002; Hunt et al., 2012). Similarly, drier conditions in the current rainforest belt of West and Central Africa favoured savannah conditions, due to a weakening of the West African Monsoon (Malhi et al., 2013).

Significantly wetter conditions persisted through the Sahara and Arabian Peninsula caused by the North African summer monsoon. However, the extent of wetter conditions has been variable during the late Pleistocene (Jennings et al., 2015). The Late Pleistocene and Holocene experienced interglacial conditions ~130ka, glacial conditions peaking ~21ka, followed by warming conditions through the Holocene, peaking ~6ka, and persisting until the present day (Braconnot et al., 2007). During the Late Pleistocene and Holocene, African and Arabian humid periods have occurred at the Last Interglacial ~130ka, ~56ka, ~21ka, and

~8ka, as characterised by a well-connected series of lakes and rivers; punctuated by hyper-arid conditions similar to those found today (Drake et al., 2011; Larrasoaña et al., 2013; Migliore et al., 2013; Jennings et al., 2015; Tierney et al., 2017).

In addition to the cyclical climate forcings of the Quaternary, the Late Pleistocene environment has been regionally affected by large-scale stochastic events, such as the super-volcanic eruption of Mt. Toba in Sumatra ~73ka. Widespread and prolonged environmental changes have been attributed to this eruption (Rampino & Self, 1992; Harris, 2008; Robock et al., 2009; Williams et al., 2009), with far-reaching consequences such as a potential bottleneck in the human population (Ambrose, 2003).

Modern humans and their ancestors have had an increasingly measureable environmental impact during the Quaternary. The impact of hominids on large carnivore guilds in Africa may have commenced over two million years ago through changes in dietary strategies leading to intraguild competition (Werdelin & Lewis, 2013). Archaic human species such as Neanderthals and Denisovans, were also present within Eurasia before the Last Interglacial (Stewart J.R. & C.B., 2012). Modern humans spread from East Africa into the Middle East ~50-60ka, Eurasia and Australasia ~35-45ka, and the Americas ~15ka (Henn et al., 2012). The sudden increase in hunting pressure specifically of large, K-selected mammalian megafauna, likely contributed to Quaternary megafaunal extinctions and subsequent, often dramatic changes in faunal composition (Koch & Barnosky, 2006; Louys et al., 2007; Barnosky & Lindsey, 2010; Stuart, 2015).

Faunal response to Quaternary instability

The widespread megafaunal extinctions of the Quaternary where species have been unable to persist in face of changing environments have been attributed to, at least in part to the climatic and environmental shifts of glacial-interglacial cycles combined with human impacts on all continents except Antarctica (Stuart, 2015). The woolly rhinoceros (*Coelodonta antiquitatis*) and cave lion (*Panthera spelaea*) did not survive the warming climate, and ingress of more woody habitats in Northern Eurasia during the Holocene (Lorenzen et al., 2011; Stuart & Lister, 2011). Southward latitudinal shifts in tropical vegetation are attributed to range reductions and subsequent extinction of *Gigantopithecus* in South East Asia (Louys

et al., 2007). Sumatran extinctions of the late Pleistocene have been attributed to the changing climate in addition to the volcanic super-eruption of Mt. Toba (Wilting et al., 2012). Changes in the extent of grasslands in Africa resulted in the loss of multiple large grazers and grassland specialists in the Holocene (Faith, 2014).

The synchronous effects of human expansion and changing climate may have exacerbated impacts on megafaunal communities (Koch & Barnosky, 2006; Heller et al., 2012), as found in South America, where the combination of late human incursions and settlement combined with climate change led to the loss of more genera in the Quaternary megafaunal extinction than on any other continent (Barnosky & Lindsey, 2010). It is notable that for sub-Saharan Africa, where megafaunal communities and species of *Homo* co-evolved, and South East Asia, where human incursions began very early, both exhibit relatively low rates of extinction during the late Quaternary (Stuart, 2015). It is likely that Late Pleistocene and early Holocene extinctions in Africa were predominantly driven by climate (Faith, 2014). Where behaviourally modern humans spread into new continents rapidly, people were at least in part responsible for the extinction of the Eurasian steppe bison (*Bison priscus*), wild horse (*Equus ferus*) in northern Eurasia (Lorenzen et al., 2011) and the local extinction of the spotted hyaena (*Crocuta crocuta*) in southern Europe (Varela et al., 2010).

Extant species have survived turbulent environmental changes by enduring within isolated refugia, or by being suitably adaptable to change. Whilst the spotted hyaena was extirpated from Europe during the Last Glacial Maximum (LGM) ~21ka, it persisted within African refugia, in part due to its broad climatic tolerance (Varela et al., 2010; Sheng et al., 2014). More specialised species have persisted within particularly resistant environments to change, such as the snow leopard (*Panthera uncia*) within the Tibetan Plateau (Li et al., 2016). Dipterocarp rainforest refugia persisted through glacial periods in Sundaland (Meijaard, 2003; Raes et al., 2014). The orangutan (*Pongo* spp.), given their arboreal lifestyle, were restricted to Sumatran and Bornean forests from a more widespread range across South East Asia (Louys et al., 2007).

The changing ranges and population dynamics of fauna through the Quaternary have dictated the recent evolutionary histories of species. The changing environmental conditions and subsequent separate, isolated refugia, and local extinction events, may have led to incipient speciation, although beetle populations in Northern Europe exhibit high evolutionary stasis

(Coope, 2004). This is in part due to the large-scale movements of populations which flood the gene pools of temporarily isolated refugia - species that have survived until now are those which are geographically mobile and latitudinally independent (Coope, 2004). A lack of phylogeographical pattern has been found in European megafauna sampled before the LGM, suggesting that the occurrence of phylogenetic patterns in current populations is an artefact of the lack of dispersal time/opportunity from refugia since the LGM following chance survival of particular genotypes within refugia (Hofreiter et al., 2004). A similar pattern is found within North Africa, where high connectivity during pluvials has limited the pattern of genetic diversity between now isolated populations of an evergreen shrub (Migliore et al., 2013). Conversely savannah adapted ungulates within sub-Saharan Africa exhibit divergence beyond the most recent pluvial/interpluvial cycles, suggesting long-term barriers to population mixing, with a limited admixture zone in East Africa (Lorenzen et al., 2012). The common toad (*Bufo bufo*) species complex is similarly split by the enduring arid conditions of central Asia through the Quaternary.

There is a clear impetus to comprehend long-term, unstable biogeographical trends to better understand the genetic and morphological variation found within extant populations of a species. The nature of morphological variation can be better understood in relation to long- and short-term vicariance, versus individual variation through phenotypic plasticity in response to prevailing environmental conditions. Top carnivores, such as big cats, provide an informative focus because of their former and present continental-scale distributions, fragmented current populations, declining numbers, ambiguous sub-specific status, key role within food webs and ecosystems, and being recipients of high levels of population management and conservation action.

Lion and Tiger Taxonomy, Subspecies and Evolutionary Significant Units

Currently the tiger ranges across Southeast Asia, Northeast Asia and the Indian Subcontinent are in increasingly isolated populations (Goodrich et al., 2015). The lion is found across sub-Saharan Africa, and within a small pocket of the Gir Forest in Northwest India. Historically, the ranges of the tiger and lion were considerably larger, more contiguous, and both species were sympatric in the Indian subcontinent. Notably, the tiger has been extirpated from the

Caspian Sea region, the islands of Bali and Java, and large swathes of mainland Asia, such as China (Goodrich et al., 2015). The lion was historically prevalent in South Africa, North Africa, the Near East and more widespread within the Indian Subcontinent and South West Asia (Bauer et al., 2016).

A sparse fossil record shows that both the lion and the tiger ranged over a geographical area not seen in historical times. The sister taxa of the modern lion ranged across North America and Northern Eurasia (Barnett et al., 2009). The cave lion is considered a distinct species, *Panthera spelaea*, from the modern lion, given its morphological and genetic distinctiveness (Stuart & Lister, 2011; Barnett et al., 2016). This closely related species declined and became extinct across Eurasia ~14ka due to the spread of shrubs and trees causing the loss of open habitats (Stuart & Lister, 2011), which may have similarly affected the modern lion in the Near East and Indian Subcontinent. Within Africa and the Near-East the changing pluvial/interpluvial cycles have likely played a role in facilitating, and limiting lion range through the expansion and contraction of dense tropical forest at low latitudes, and cycles of hyper aridfication of North Africa and the Near-East (Larrasoaña et al., 2013; Malhi et al., 2013; Jennings et al., 2015; Tierney et al., 2017). In addition to fossil evidence during the Quaternary of the tiger within its recent historical range, there is also evidence of it on the now isolated islands of Borneo, Sri Lanka and Japanese Archipelago (Kitchener & Yamaguchi, 2010). The changing forest cover across Asia during the Pleistocene, in addition to fluctuations in sea levels has likely played a key role in determining the Quaternary range patterns of the tiger.

Extant species of the genus *Panthera* likely diverged from one another during the Late Miocene (Tseng et al., 2014). However, variation within each species can be attributed to more recent times. Subspecies are not necessarily objective concepts, at least not to the extent to which species are entities (Groves, 2012), but the notion of subspecies is still biologically meaningful, as subspecies should represent unique evolutionary lineages. Therefore, it is necessary for successful conservation management to understand subspecies definitions (Groves, 2012), as subspecies are often used as proxy units of conservation (Zink, 2004). Phylogenetic studies can delineate clades and subspecies within each population as a whole. To an extent these studies can identify trends in population size (e.g. Heller et al., 2012; Bertola et al., 2016), and divergence times between clades, the findings of which allude to the biogeographical drivers of change. The tiger is composed of two putative subspecies, the

Sunda tiger (*Panthera tigris sondaica*), and the continental tiger (*Panthera tigris tigris*) based upon molecular, morphological and ecological data (Wilting et al., 2015). The data suggest a population collapse consistent with the super-Eruption of Mt. Toba, and the split between subspecies coinciding with the elimination of land bridges between the Sunda Islands and mainland Asia following the LGM (Kitchener & Dugmore, 2000; Wilting et al., 2015). However, recognition of two subspecies in the tiger is relatively new, and still contested by a greater number of traditionally recognised groups (see Goodrich et al., 2015; Liu et al., 2018). Genome wide analysis provides a powerful tool to differentiate between populations (see Liu et al., 2018), however, the tiger has undergone significant recent population bottlenecks in increasingly isolated fragments of its former range, which may hamper the ability to identify the genetic basis of adapatation with genomic data (Crisci et al., 2016). The lion has also undergone recent revision of subspecific groupings, with recognition of a northern (*Panthera leo leo*; West Africa, Central Africa and North Africa/Asia) and southern (*P.l.Melanochaita*; North East, East/Southern and South West) subspecies (Barnett et al., 2014; Bertola et al., 2016). It is proposed that the distinct clades were formed through long term vicariance by semi permeable environmental barriers in East Africa, such as the Rift Valley (Bertola et al., 2016).

Whilst the power of genetic analyses are clear, morphological studies still provide perspectives on genetic expression, and the functional diversity of different clades and clines (Cardini, 2003; Cardini et al., 2007). Studies of big cat morphology have been used to differentiate (see Mazák & Groves, 2006; Christiansen & Harris, 2009; Mazák, 2010; Yamaguchi et al., 2013) or show similarities (see Kitchener, 1999; Mazák, 2010; Yamaguchi et al., 2013; Wilting et al., 2015) between populations of big cats using a range of statistical techniques applied to skull metrics. An insight into the variation in the morphology of big cats across their geographical range, and environmental conditions, rather than explicitly focussing on categorical groups, could increase understanding of both of the nature of apparent morphological variation, and the historical/environmental processes from which variation may have arisen.

Challenges

Investigating the shifting ranges of big cats through deep time, and understanding their morphologies throughout their ranges is problematic on three fronts a) The nature of subspecific variation is potentially driven by tens of thousands of years of environmental change. b) The potential ranges of the lion and tiger span continental scales. c) There is a lack of or biased samples for investigation.

Historical accounts have little value in determining the long-term Quaternary ranges of lions and tigers. Patchy fossil records for the lion and tiger discount the use of widespread spatially explicit empirical evidence for each species' continuous range. Genetic divergence times and population skyline plots give some insight, yet are not spatially explicit, and often have wide confidence intervals, which do not adequately constrain events in time. Whilst changing environments have dictated the changing ranges of lions and tigers, climatic and environmental data that are continuous in space across continental ranges are not available in near continuous time over the Quaternary. When continuous climatic data are available, for example, the mid-Holocene and Last Glacial Maximum, the accuracy of these models can be highly variable (e.g. Tierney et al., 2017)

The rising anthropogenic pressure upon both lions and tigers can cause problems through population fragmentation and founder effects. These impacts present challenges to understanding; modern distributions and populations may be a fraction of those of the past; and thus incomplete sampling, large-scale range shifts and local extirpation can obscure patterns, and make inferences of past distributions difficult or prone to misinterpretation. Therefore phylogenetic studies may be of limited utility due to the recent extinction of genetic variants (Weisrock & Janzen, 2000; Winker, 2010), but similarly impact morphological and biogeographical studies, where morphological variation and environmental niches are not represented. Human-induced translocations may also affect genetic studies (Bertola et al., 2016), especially when sample sizes are small.

In addition to issues surrounding fragmented ranges, the understanding of morphological variation is affected by changes to individuals throughout their development. Mechanical stresses and nutritional intake can influence the phenotype of individuals through

development (Smuts et al., 1978; Mowat & Heard, 2006), and the phenotypic plasticity of bone may provide an ecological advantage by allowing for the full size range that a morphotype can occupy in response to local environmental conditions (Doube et al., 2009). It is important to understand the extent to which morphological traits are dictated by the environment of an individual, and which traits are controlled by an individual's genes and evolutionary history.

This research is framed in recognition of these challenges, which highlight the limitations of a genetics-only approach, and identify obstacles to overcome from both biogeographical modelling and morphological analyses.

Research Topics

Biogeographical modelling of the lion and the tiger

The shifting ranges of both the lion and tiger are often interpreted from phylogenetic analyses and literature reviews of environmental conditions (Driscoll et al., 2009; Luo et al., 2014; Wilting et al., 2015; Bertola et al., 2016). Where spatially explicit modelling has occurred (e.g. Kitchener & Dugmore, 2000; Townsend Peterson et al., 2014), the data and methods are outdated, or are not projected onto modelled climates of the past.

Research is needed to model the palaeo-distributions and changing ranges of both the lion and tiger, which accounts for the uncertainty in palaeo-environmental data and uses up-to-date or novel techniques, which aid in the interpretation of modelled outputs. It is important to consider additional drivers of change, such as super-volcanic eruptions, non-climatic anthropogenic impacts, and competing megafauna, to allow a holistic, spatially explicit examination of the changing population range size, vicariance and contiguity throughout recent evolutionary time. These biogeographical analyses will contextualise existing genetic and morphological literature on both species, by framing the evolutionary drivers of current variation, and frame the more thorough morphological analysis which forms the second facet of this research.

Morphological analysis of the lion and the tiger

A species' morphology is an integral part of understanding subspecific taxonomy (Torstrom et al., 2014). Multiple studies have analysed lion and tiger skull morphology in relation to taxonomy and geographical origin (Mazák & Groves, 2006; Barnett et al., 2008; Kitchener & Yamaguchi, 2010; Mazák, 2010; Yamaguchi et al., 2013; Wilting et al., 2015), whilst others have assessed the effects of captivity upon felid skull morphology (Hollister, 1917; Duckler, 1998; Zuccarelli, 2004; O'Regan & Kitchener, 2005; Hartstone-Rose et al., 2014; Saragusty et al., 2014). Morphological studies of wild specimens have overlooked the potential influence of phenotypic plasticity upon individuals, whereas studies which examine the effects of captivity have not adequately addressed the implications for variation in wild populations. There is a need to consider a wide range of morphological variables, covering specimens from the known historic extents of each species, in relation to phenotypic plasticity, geographical origin, and recent evolutionary history.

Research Questions

The key questions relating to biogeographical change, and morphological expression in the lion and tiger are identified here, which will be addressed in this thesis.

1. **Biogeographical change in the tiger and lion:** *What is the degree of natural connectivity between currently separated lion and tiger populations, both for the present and Late Pleistocene conditions?* This question addresses the explicit biogeographical drivers of recent evolutionary history, and contextualises current and future genetic and morphological research.

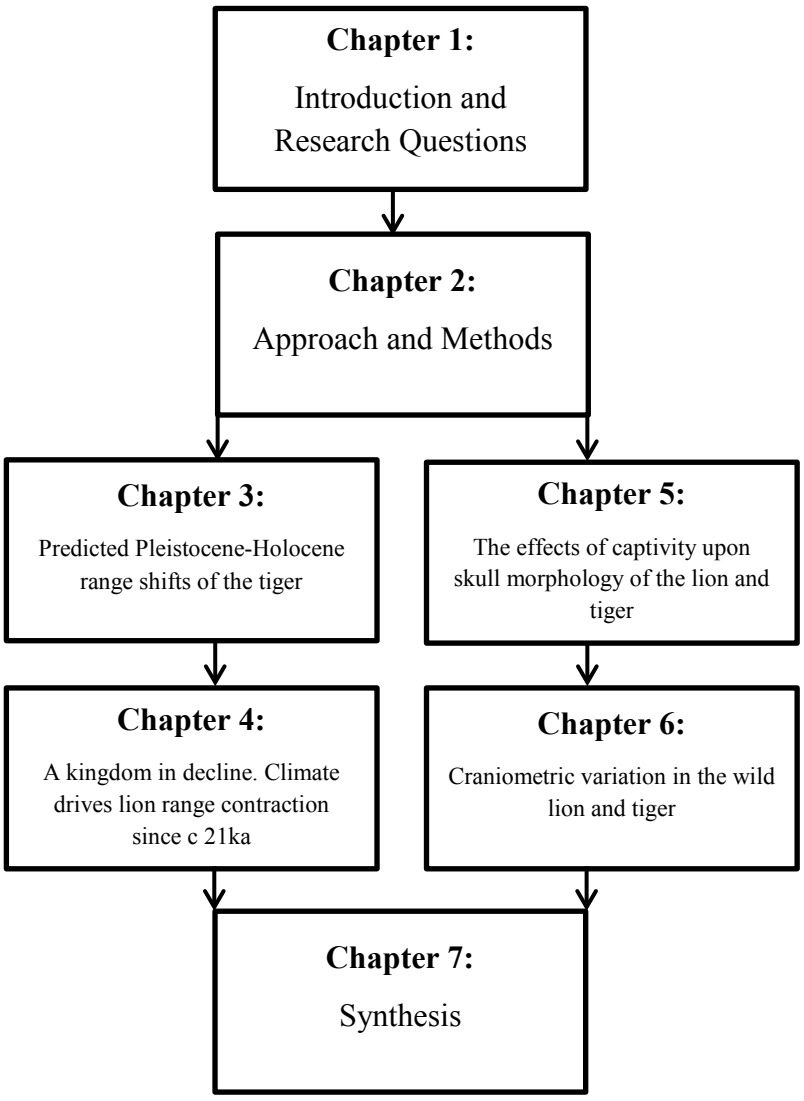
2. **Phenotypic Plasticity:** *To what extent is the skull morphology of lions and tigers determined by diet and environment?* This question highlights the need to create a methodological framework which first identifies measurements and features which are strongly affected by an individual's development. This is an important step to better understand variation in wild morphology so as to differentiate between life history and evolutionary history.

3. **Wild Morphology:** *What are the causes of morphological variation in wild lions and tigers across each species' geographical range?* This question puts lion and tiger

morphology into a geographical perspective, and brings together the drivers of evolutionary differentiation (biogeographical modelling) with the potential influence of phenotypic plasticity.

Thesis Structure

The structure of this thesis is outlined in *Figure 1*.



Chapter 2 explores the applicability of the data, models and analytical techniques available for the proposed research questions. **Chapter 3** details the biogeographical history of the tiger throughout the Late-Pleistocene and Holocene using a Maximum Entropy modelling approach in conjunction with multiple General Circulation Models of palaeo-climate. **Chapter 4** models the range shifts of the lion, and builds upon the MaxEnt methodology of

Chapter 3 by introducing a categorical framework of Global Environmental Strata and Zones to explain niche suitability. **Chapter 5** investigates the phenotypic plasticity of skull morphology in the lion and tiger, by analysing differences between captive and wild specimens, thereby representing vastly different environmental conditions. **Chapter 6** builds upon the basic methodology and results of Chapter 5 to investigate the causes of wild variation in lion and tiger skull morphology. **Chapter 7** synthesises the two strands of investigation by linking the biogeographical drivers for variation with the morphological variation found in modern populations.

References

Ambrose S.H. (2003) Did the super-eruption of Toba cause a human population bottleneck? Reply to Gathorne-Hardy and Hartcourt-Smith. *Journal of Human Evolution*, **45**, 231–237.

Barnett R., Mendoza M.Z., Soares A.R., Ho S., Zazula G., Yamaguchi N., Shapiro B., Kirillova I., Larson G., & Gilbert M. (2016) Mitogenomics of the Extinct Cave Lion, Panthera spelaea (Goldfuss, 1810), Resolve its Position within the Panthera Cats. *Open Quaternary*, **2**, 1–11.

Barnett R., Shapiro B., Barnes I., Ho S.Y.W., Burger J., Yamaguchi N., Higham T.F.G., Wheeler H.T., Rosendahl W., Sher A. V, Sotnikova M., Kuznetsova T., Baryshnikov G.F., Martin L.D., Harington C.R., Burns J.A., & Cooper A. (2009) Phylogeography of lions (Panthera leo ssp.) reveals three distinct taxa and a late Pleistocene reduction in genetic diversity. *Molecular ecology*, **18**, 1668–77.

Barnett R., Yamaguchi N., Shapiro B., Ho S.Y.W., Barnes I., Sabin R., Werdelin L., Cuisin J., & Larson G. (2014) Revealing the maternal demographic history of Panthera leo using ancient DNA and a spatially explicit genealogical analysis. *BMC Evolutionary Biology*, **14**, 70.

Barnett R., Yamaguchi N., Shapiro B., & Sabin R. (2008) Ancient DNA analysis indicates the first English lions originated from North Africa. *Contributions to Zoology*, **77**, 7–16.

Barnosky A.D. & Lindsey E.L. (2010) Timing of Quaternary megafaunal extinction in South America in relation to human arrival and climate change. *Quaternary International*, **217**, 10–29.

Bauer H., Packer C., Funston P.F., Henschel P., & Nowell K. (2016) Available at: http://www.iucnredlist.org/details/summary/15951/0.

Bertola L.D., Jongbloed H., van der Gaag K.J., de Knijff P., Yamaguchi N., Hooghiemstra H., Bauer H., Henschel P., White P.A., Driscoll C.A., Tende T., Ottosson U., Saidu Y., Vrieling K., & de Iongh H.H. (2016) Phylogeographic Patterns in Africa and High Resolution Delineation of Genetic Clades in the Lion (Panthera leo). *Scientific Reports*, **6**, 1–11.

Binney H., Edwards M., Macias-Fauria M., Lozhkin A., Anderson P., Kaplan J.O., Andreev A., Bezrukova E., Blyakharchuk T., Jankovska V., Khazina I., Krivonogov S., Kremenetski K., Nield J., Novenko E., Ryabogina N., Solovieva N., Willis K., &

Zernitskaya V. (2017) Vegetation of Eurasia from the last glacial maximum to present: Key biogeographic patterns. *Quaternary Science Reviews*, **157**, 80–97.

Braconnot P., Otto-Bliesner B., Harrison S., Joussaume S., Peterchmitt J.-Y., Abe-Ouchi a., Crucifix M., Driesschaert E., Fichefet T., Hewitt C.D., Kageyama M., Kitoh a., Laîné a., Loutre M.-F., Marti O., Merkel U., Ramstein G., Valdes P., Weber S.L., Yu Y., & Zhao Y. (2007) Results of PMIP2 coupled simulations of the Mid-Holocene and Last Glacial Maximum – Part 1: experiments and large-scale features. *Climate of the Past*, **3**, 261–277.

Cardini A. (2003) The geometry of the marmot (Rodentia: Sciuridae) mandible: Phylogeny and patterns of morphological evolution. *Systematic Biology*, **52**, 186–205.

Cardini A., Jansson A.-U., & Elton S. (2007) A geometric morphometric approach to the study of ecogeographical and clinal variation in vervet monkeys. *Journal of Biogeography*, **34**, 1663–1678.

Chevalier M., Brewer S., & Chase B.M. (2017) Qualitative assessment of PMIP3 rainfall simulations across the eastern African monsoon domains during the mid-Holocene and the Last Glacial Maximum. *Quaternary Science Reviews*, **156**, 107–120.

Christiansen P. & Harris J.M. (2009) Craniomandibular morphology and phylogenetic affinities of Panthera atrox : implications for the evolution and paleobiology of the lion lineage. *Journal of Vertebrate Paleontology*, **29**, 934–945.

Coope G.R. (2004) Several million years of stability among insect species because of, or in spite of, Ice Age climatic instability? *Philosophical Transactions of the Royal Society B: Biological Sciences*, **359**, 209–214.

Crisci J.L., Dean M.D., & Ralph P. (2016) Adaptation in isolated populations: when does it happen and when can we tell? *Molecular ecology*, **25**, 3901–3911.

Dietl G.P. & Flessa K.W. (2011) Conservation paleobiology: Putting the dead to work. *Trends in Ecology and Evolution*, **26**, 30–37.

Doube M., Wiktorowicz-Conroy A., Conroy A.W., Christiansen P., Hutchinson J.R., & Shefelbine S. (2009) Three-dimensional geometric analysis of felid limb bone allometry. *PloS one*, **4**, e4742.

Drake N.A., Blench R.M., Armitage S.J., Bristow C.S., & White K.H. (2011) Ancient watercourses and biogeography of the Sahara explain the peopling of the desert. *Proceedings of the National Academy of Sciences*, **108**, 458–462.

Driscoll C.A., Yamaguchi N., Bar-Gal G.K., Roca A.L., Luo S., Macdonald D.W., & O'Brien S.J. (2009) Mitochondrial phylogeography illuminates the origin of the extinct

caspian tiger and its relationship to the amur tiger. *PloS one*, **4**, e4125.

Duckler G.L. (1998) An unusual osteological formation in the posterior skulls of captive tigers (Panthera tigris). *Zoo Biology*, **17**, 135–142.

Faith J.T. (2014) Late Pleistocene and Holocene mammal extinctions on continental Africa. *Earth-Science Reviews*, **128**, 105–121.

Gathorne-Hardy F.J., Syaukani, Davies R.G., Eggelton P., & Jones D.T. (2002) Quaternary rainforest refugia in South-East Asia: Using termites (Isoptera) as indicators. *Biological Journal of the Linnean Society*, **75**, 453–466.

Goodrich J., Lynam A., Miquelle D., Wibisono H., Kawanishi K., Pattanavibool A., Htun S., Tempa T., Karki J., Jhala Y., & Karanth U. (2015) Available at: www.iucnredlist.org.

Groves C. (2012) Species Concept in Primates. *American Journal of Primatology*, **74**, 687–691.

Harris B. (2008) The potential impact of super-volcanic eruptions on the Earth's atmosphere. *Weather*, **63**, 221–225.

Hartstone-Rose A., Selvey H., Villari J.R., Atwell M., & Schmidt T. (2014) The Three-Dimensional Morphological Effects of Captivity. *PLoS ONE*, **9**, e113437.

Heller R., Brüniche-Olsen A., & Siegismund H.R. (2012) Cape buffalo mitogenomics reveals a Holocene shift in the African human-megafauna dynamics. *Molecular Ecology*, **21**, 3947–3959.

Henn B.M., Cavalli-Sforza L.L., & Feldman M.W. (2012) The great human expansion. *Proceedings of the National Academy of Sciences*, **109**, 17758–17764.

Hofreiter M., Serre D., Rohland N., Rabeder G., Nagel D., Conard N., Munzel S., & Paabo S. (2004) Lack of phylogeography in European mammals before the last glaciation. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 12963–12968.

Holland K.K., Larson L.R., & Powell R.B. (2018) Characterizing conflict between humans and big cats Panthera spp: A systematic review of research trends and management opportunities. *PLoS ONE*, **13**, 1–19.

Hollister N. (1917) Some effects of environment and habit on captive lions. *Proceedings of the US National Museum*, **53**, 177–193.

Hunt C.O., Gilbertson D.D., & Rushworth G. (2012) A 50,000-year record of late Pleistocene tropical vegetation and human impact in lowland Borneo. *Quaternary Science Reviews*, **37**, 61–80.

Imbrie J., Boyle E.A., Clemens S.C., Duffy A., Howard W.R., Kukla G., Kutzbach J.,

Martinson D.G., Mcintyre A., Mix A.C., Molfino B., Morley J.J., Peterson L.C., Pisias N.G., Prell W.L., Raymo M.E., Shackleton N.J., & Toggweiler J.R. (1992) On the structure and origin of major glaciation cycles. 1. Linear responses to Milankovitch forcing. *Paleoceanography*, **7**, 701–738.

Imbrie J. & Imbrie K.P. (1979) *Ice ages: solving the mystery.* Macmillan, London.

Jennings R.P., Singarayer J., Stone E.J., Krebs-Kanzow U., Khon V., Nisancioglu K.H., Pfeiffer M., Zhang X., Parker A., Parton A., Groucutt H.S., White T.S., Drake N.A., & Petraglia M.D. (2015) The greening of Arabia: Multiple opportunities for human occupation of the Arabian Peninsula during the Late Pleistocene inferred from an ensemble of climate model simulations. *Quaternary International*, **382**, 181–199.

Karl S. a. & Bowen B.W. (1999) Evolutionary Significant Units versus Geopolitical Taxonomy: Molecular Systematics of an Endangered Sea Turtle (genus Chelonia). *Conservation Biology*, **13**, 990–999.

Kitchener A.C. (1999) Tiger Distribution, Phenotypic Variation and Conservation Issues. *Riding the Tiger. Tiger Conservation in Human-Dominated Landscapes* (ed. by J. Seidensticker, J. Christie, and P. Jackson), pp. 19–39. Cambridge University Press, Cambridge.

Kitchener A.C. & Dugmore A.J. (2000) Biogeographical change in the tiger , Panthera tigris. *Animal Conservation*, **3**, 113–124.

Kitchener A.C. & Yamaguchi N. (2010) What is a tiger? Biogeography, morphology, and taxonomy. *Tigers of the World* (ed. by R. Tilson and P. Nyhus), pp. 53–84. Academic Press, London.

Koch P.L. & Barnosky A.D. (2006) Late Quaternary extinctions: State of the debate. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 215–250.

Larrasoaña J.C., Roberts A.P., & Rohling E.J. (2013) Dynamics of Green Sahara Periods and Their Role in Hominin Evolution. *PLoS ONE*, **8**, .

Li J., McCarthy T.M., Wang H., Weckworth B. V., Schaller G.B., Mishra C., Lu Z., & Beissinger S.R. (2016) Climate refugia of snow leopards in High Asia. *Biological Conservation*, **203**, 188–196.

Liu Y.-C., Sun X., Driscoll C., Miquelle D.G., Xu X., Martelli P., Uphyrkina O., Smith J.L.D., O'Brien S.J., & Luo S.-J. (2018) Genome-Wide Evolutionary Analysis of Natural History and Adaptation in the World's Tigers. *Current Biology*, **28**, 3840–3849.e6.

Lorenzen E.D., Heller R., & Siegismund H.R. (2012) Comparative phylogeography of

African savannah ungulates. *Molecular Ecology*, **21**, 3656–3670.

Lorenzen E.D., Nogués-Bravo D., Orlando L., Weinstock J., Binladen J., Marske K. a, Ugan A., Borregaard M.K., Gilbert M.T.P., Nielsen R., Ho S.Y.W., Goebel T., Graf K.E., Byers D., Stenderup J.T., Rasmussen M., Campos P.F., Leonard J. a, Koepfli K.-P., Froese D., Zazula G., Stafford T.W., Aaris-Sørensen K., Batra P., Haywood A.M., Singarayer J.S., Valdes P.J., Boeskorov G., Burns J. a, Davydov S.P., Haile J., Jenkins D.L., Kosintsev P., Kuznetsova T., Lai X., Martin L.D., McDonald H.G., Mol D., Meldgaard M., Munch K., Stephan E., Sablin M., Sommer R.S., Sipko T., Scott E., Suchard M. a, Tikhonov A., Willerslev R., Wayne R.K., Cooper A., Hofreiter M., Sher A., Shapiro B., Rahbek C., & Willerslev E. (2011) Species-specific responses of Late Quaternary megafauna to climate and humans. *Nature*, **479**, 359–64.

Louys J., Curnoe D., & Tong H. (2007) Characteristics of Pleistocene megafauna extinctions in Southeast Asia. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **243**, 152–173.

Luo S.-J., Zhang Y., Johnson W.E., Miao L., Martelli P., Antunes A., Smith J.L.D., & O'Brien S.J. (2014) Sympatric Asian felid phylogeography reveals a major Indochinese-Sundaic divergence. *Molecular ecology*, **23**, 2072–92.

Malhi Y., Adu-bredu S., Asare R.A., Lewis S.L., & Mayaux P. (2013) African rainforests : past , present and future. *Philosophical Transactions of the Royal Society B*, **368**, 1–10.

Mazák J.H. (2010) Craniometric variation in the tiger (Panthera tigris): Implications for patterns of diversity, taxonomy and conservation. *Mammalian Biology - Zeitschrift für Säugetierkunde*, **75**, 45–68.

Mazák J.H. & Groves C.P. (2006) A taxonomic revision of the tigers (Panthera tigris) of Southeast Asia. *Mammalian Biology*, **71**, 268–287.

Meijaard E. (2003) Mammals of south-east Asian and their Late Pleistocene environment. *Journal of Biogeography*, 1245–1257.

Migliore J., Baumel A., Juin M., Fady B., Roig A., Duong N., & Médail F. (2013) Surviving in Mountain Climate Refugia: New Insights from the Genetic Diversity and Structure of the Relict Shrub Myrtus nivellei (Myrtaceae) in the Sahara Desert. *PLoS ONE*, **8**, 1–10.

Mowat G. & Heard D.C. (2006) Major components of grizzly bear diet across North America. *Canadian Journal of Zoology*, **84**, 473–489.

O'Regan H.J. & Kitchener A.C. (2005) The effects of captivity on the morphology of captive, domesticated and feral mammals. *Mammal Review*, **35**, 215–230.

Raes N., Cannon C.H., Hijmans R.J., Piessens T., Saw L.G., van Welzen P.C., & Slik J.W.F. (2014) Historical distribution of Sundaland's Dipterocarp rainforests at Quaternary

glacial maxima. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 16790–5.

Rampino M.R. & Self S. (1992) Volcanic winter and accelerated glaciation following the Toba super-eruption. *Nature*, **359**, 50–52.

Rebelo H., Froufe E., Brito J.C., Russo D., Cistrone L., Ferrand N., & Jones G. (2012) Postglacial colonization of Europe by the barbastelle bat: agreement between molecular data and past predictive modelling. *Molecular Ecology*, **21**, 2761–74.

Robock A., Ammann C.M., Oman L., Shindell D., Levis S., & Stenchikov G. (2009) Did the Toba volcanic eruption of ~74 ka B.P. produce widespread glaciation? *Journal of Geophysical Research*, **114**, D10107.

Rohling E.J., Fenton M., Jorissen F.J., Bertrand P., Ganssen G., & Caulet J.P. (1998) Magnitudes of sea-level lowstands of the past 500,000 years. *Nature*, **394**, 162–165.

Saragusty J., Shavit-Meyrav A., Yamaguchi N., Nadler R., Bdolah-Abram T., Gibeon L., Hildebrandt T.B., & Shamir M.H. (2014) Comparative skull analysis suggests species-specific captivity-related malformation in lions (Panthera leo). *PloS one*, **9**, e94527.

Sheng G.L., Soubrier J., Liu J.Y., Werdelin L., Llamas B., Thomson V.A., Tuke J., Wu L.J., Hou X.D., Chen Q.J., Lai X.L., & Cooper A. (2014) Pleistocene Chinese cave hyenas and the recent Eurasian history of the spotted hyena, Crocuta crocuta. *Molecular Ecology*, **23**, 522–533.

Smuts G.L., Anderson J.L., & Austin J.C. (1978) Age determination of the African lion (Panthera leo). *Journal of Zoology*, **185**, 115–146.

Stewart J.R. & C.B. S. (2012) Human Evolution Out of Africa: The Role of Refugia and Climate Change. *Science*, **335**, 317–321.

Stuart A.J. (2015) Late Quaternary megafaunal extinctions on the continents: a short review. *Geological Journal*, **50**, 338–363.

Stuart A.J. & Lister A.M. (2011) Extinction chronology of the cave lion Panthera spelaea. *Quaternary Science Reviews*, **30**, 2329–2340.

Tierney J.E., Pausata F.S.R., & DeMenocal P.B. (2017) Rainfall regimes of the Green Sahara. *Science Advances*, **3**, e1601503.

Torstrom S.M., Pangle K.L., & Swanson B.J. (2014) Shedding subspecies: The influence of genetics on reptile subspecies taxonomy. *Molecular Phylogenetics and Evolution*, **76**, 134–143.

Townsend Peterson A., Radocy T., Hall E., Kerbis Peterhans J.C., & Celesia G.G. (2014) The potential distribution of the Vulnerable African lion Panthera leo in the face of changing

global climate. *Oryx*, **48**, 1–10.

Tseng Z.J., Wang X., Slater G.J., Takeuchi G.T., Li Q., Liu J., & Xie G. (2014) Himalayan fossils of the oldest known pantherine establish ancient origin of big cats. *Proc Biol Sci*, **281**, 20132686.

Varela S., Lobo J.M., Rodríguez J., & Batra P. (2010) Were the Late Pleistocene climatic changes responsible for the disappearance of the European spotted hyena populations? Hindcasting a species geographic distribution across time. *Quaternary Science Reviews*, **29**, 2027–2035.

Wang X., Sun X., Wang P., & Stattegger K. (2009) Vegetation on the Sunda Shelf, South China Sea, during the Last Glacial Maximum. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **278**, 88–97.

Weisrock D.W. & Janzen F.J. (2000) Comparative Molecular Phylogeography of North American Softshell Turtles (Apalone): Implications for Regional and Wide-Scale Historical Evolutionary Forces. *Molecular Phylogenetics and Evolution*, **14**, 152–164.

Werdelin L. & Lewis M.E. (2013) Temporal Change in Functional Richness and Evenness in the Eastern African Plio-Pleistocene Carnivoran Guild. *PLoS ONE*, **8**, 1–11.

Williams M.A.J., Ambrose S.H., van der Kaars S., Ruehlemann C., Chattopadhyaya U., Pal J., & Chauhan P.R. (2009) Environmental impact of the 73ka Toba super-eruption in South Asia. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **284**, 295–314.

Wilting A., Courtiol A., Christiansen P., Niedballa J., Scharf A.K., Orlando L., Balkenhol N., Hofer H., Kramer-Schadt S., Fickel J., & Kitchener A.C. (2015) Planning tiger recovery: Understanding intraspecific variation for effective conservation. *Science Advances*, **1**, 1–13.

Wilting A., Sollmann R., Meijaard E., Helgen K.M., & Fickel J. (2012) Mentawai's endemic, relictual fauna: Is it evidence for Pleistocene extinctions on Sumatra? *Journal of Biogeography*, **39**, 1608–1620.

Winker K. (2010) Subspecies Represent Geographically Partitioned Variation, A Gold Mine of Evolutionary Biology, and a Challenge for Conservation. *Ornithological Monographs*, 6–23.

Yamaguchi N., Driscoll C.A., Werdelin L., Abramov A. V, Cuisin J., Fernholm B., Hiermeier M., Hills D., Hunter L., Johansson U.S., Kascheev V., Krohmann K., Martin T., Pavlinov I.Y., Renoud F., Tomsett L., Mije S. Van Der, Groves C., Kitchener A.C., Nijman V., & Macdonald D.W. (2013) Locating Specimens of Extinct Tiger (Panthera tigris) Subspecies: Javan Tiger (P.T. sondaica), Balinese Tiger (P.T. balica), and

Caspian Tiger (P.T. virgata), Including Previously Unpublished Specimens. *The Mammal Society of Japan*, **38**, 187–198.

Zink R.M. (2004) The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proceedings of the Royal Society B*, **271**, 561–4.

Zuccarelli M.D. (2004) Comparative morphometric analysis of captive vs. wild African lion (Panthera leo) skulls. *Bios*, **75**, 131–138.

Chapter 2: Approach and Methods

Introduction

This chapter provides an overview of the methodological decisions which are expanded upon in *Chapters 3-6,* which independently present the methods utilised to address the research questions of this thesis. The methodological implications that connect individual chapters and the overall research themes of this thesis are discussed herein

Scales of Analysis

Divergence events in the genus *Panthera* originate in the Late Miocene (Tseng et al., 2014), yet subspecific diversity has occurred at much more recent timescales. Genetic differentiation within the lion and tiger ranges from tens of thousands to hundreds of thousands of years (Wilting et al., 2015; Bertola et al., 2016) which covers the mid- to Late Pleistocene and Holocene. The lion and tiger are both generalist apex predators within their broad respective niches (Eloff, 1973; Nowell & Jackson, 1996; Kanagaraj et al., 2011), and as such both species have historically occupied continental scale ranges within Africa and Asia. This thesis is therefore concerned with broad spatio-temporal scales to assess subspecific biogeographical patterns which could be considered as coarse in relation to landscape ecology, or when compared to less mobile, geographically constrained specialist species. In the context of evolutionary history however, exploring patterns of subspecific variation is very recent, and evolutionary differences are comparatively small. It is important to explore spatially explicit biogeographical patterns, which may explain variation at continental scales, with consideration for the nature of subspecific allopatry. At such fine evolutionary scales, morphological variation across the range of each species may be as equally affected by the life histories of individuals as by differentiation through evolutionary divergence. This chapter outlines the broad methodological approaches to answering the research questions proposed in *Chapter 1*, with consideration for the spatio-temporal scales of variation and of the data available.

Biogeographical Modelling

Species Distribution Modelling

Species Distribution Models, or Ecological Niche Models, associate known point locations of a taxon, with a set of relevant environmental variables to create a modelled niche (*Figure 1*). The variables used may directly affect the species (e.g. climatic extremes, or the density of preferred prey), or may be climate proxies which determine the floral and faunal assemblage which supports a species. The environmental variables should affect the species' distribution at the relevant scale, determined by the geographical extent and grain of the modelling task (Pearson et al., 2004).

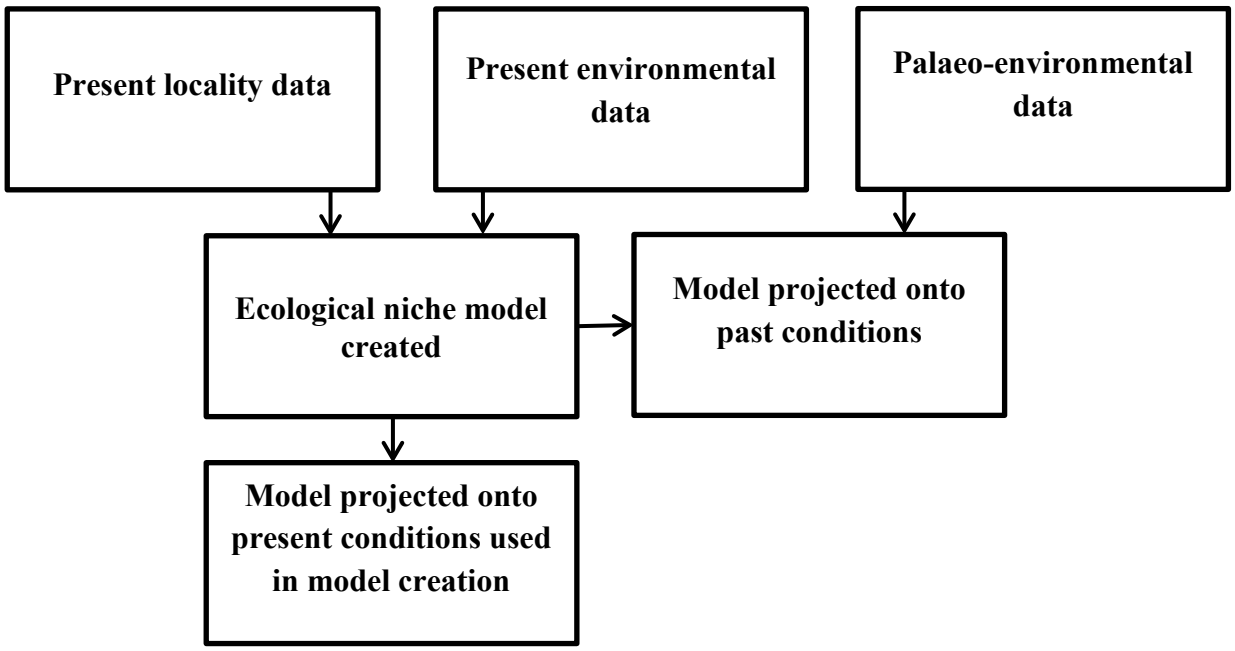


***Figure 1*:** The basic premise of the ecological niche modelling utilised by *Chapters 3 + 4* to discern tiger and lion ranges for the present day and the Late Pleistocene.

Ecological Niche Modelling scenario maps are a useful complement to molecular studies, offering a less subjective spatially explicit hypothesis of past geographical patterns of distribution (Waltari et al., 2007). Niche modelling techniques have become common in recent phylogeographical analysis (Alvarado-Serrano & Knowles, 2014), and provide useful evidence of allopatry between populations (Raxworthy et al., 2007). Identifying Last Glacial Maximum (LGM) refugia from ecological niche models has been shown to correlate strongly

with phylogeographic analyses, and provides a less subjective, spatially explicit result (Waltari et al., 2007; Kozak et al., 2008).

Climate is a key factor in defining ecological niches and the geographical distribution of species at continental scales (Geffen et al., 2004; Varela et al., 2010), and has been used to model megafaunal ranges during previous timescales (Varela et al., 2010; Lorenzen et al., 2011). Niche models comparing recent and fossil locality records, have shown that mammal species have tracked consistent climate profiles from the LGM (Martínez-Meyer et al., 2004) and therefore climate change may be used to infer mammal range changes.

Basic needs of the lion and the tiger

Tigers require forest or woodland cover, sufficient large ungulate prey and access to water (Nowell & Jackson, 1996). Kitchener & Dugmore, (2000) focused on vegetation zones, topography and precipitation as constraints to tiger distribution through their impact on prey species, whilst Kanagaraj et al., (2011) incorporated prey-species localities directly into tiger distribution models. Kanagaraj et al., (2011) found almost binary tiger habitat suitability in Nepal, with a tiger habitat preference for dense forest with that of prey species and very low tiger occurrence outside these conditions. Lions have a broad habitat tolerance, with optimal habitat comprising of open woodland and thick bush, scrub and grass complexes (Nowell & Jackson, 1996). However, they are able to survive in very arid environments (Eloff, 1973). Lions are likely to have evolved group-living behaviour before they expanded out of Africa and before the Late Pleistocene (Yamaguchi et al., 2004) and therefore the ecological niche of lions is unlikely to have changed dramatically due to changes in social structure over the time period of interest for ecological niche modelling.

Lion and tiger ranges have historically overlapped, although within separate niches, with the lion adapted to dry open savannah and the tiger to forests (Sunquist, 1981). However, it is expected that lions could limit tiger distributions in areas of niche overlap due to the lion's group-living behaviour. Other than the lion, the tiger's large size means that its only potential competitor is the dhole, *Cuon alpinus* which is a canid (Sunquist, 1981). With both lions and tigers as the dominant predators within their respective ranges, intraguild competition has

likely been negligible throughout the Late Pleistocene and is therefore unlikely to have affected each species' niche, except for where their ranges overlap.

Data repositories and quality

Species locality points used within the modelling process are collected from multiple sources from online databases, published research, and museum record information. By using a wide range of resources, the maximum number of high quality locality records can be utilised, and the influence of spatially autocorrelated localities from single sources is reduced. This research is interested in long term trends in lion and tiger range, and therefore this analysis can utilise historical records of now extirpated populations from hundreds of years ago, as these represent a species' preference for broadly similar climatic conditions to those found today. The recognition of spatial error in locality data is an important area for research in ecological niche modelling that has not been well investigated (Raxworthy et al., 2007) however given the large home-range sizes of both lions and tigers and the continental scale of these analyses, issues of locality error are somewhat mitigated.

Bioclimatic datasets are available through, and derived from, the WorldClim data (Hijmans et al., 2005; Zomer et al., 2007, 2008; Metzger et al., 2013). Data are available for multiple Coupled General Circulation Models (CGCM's) for different time slices, and at different spatial resolutions. Categorical environmental data is not utilised in the initial model creation reported within this thesis because they can decrease model performance in comparison to models created based on bioclimatic variables (Martínez-Meyer et al., 2004). The use of categorical variables in the creation of ecological niche models increases the opportunity for the misclassification of suitable environmental conditions based on locality data error in comparison with continuous variables. However, categorical bioclimatic variables can be useful in the projection of models to novel environments by providing a more descriptive framework, as outlined in *Chapter 5*.

The density of lion and tiger populations is an important consideration in relation to the resolution of environmental variables utilised at continental scales. Ecological niche theory is used as the basis of species distribution modelling (Phillips et al., 2006), and was envisaged at community scales. For models created at continental scales, with coarse-resolution data,

species competing for the same resource may well co-occur within the same grid cell (Araújo & Guisan, 2006). The resolution of datasets will affect how well the environmental variable resolution corresponds with home-range size and distribution patterns. The range of habitat sizes across the global tiger and lion distributions is large. Kanagaraj et al., (2011) identify a critical neighbourhood of 37km^2 for female tiger home ranges in Nepal. However, Rapoport's Rule (Stevens, 1989) determines that individuals at higher latitudes will have larger home ranges than individuals at lower latitudes. Whilst a 2° resolution model may be appropriate for tigers within northern latitudes, where home ranges are vast, this is less appropriate within the Sunda Islands, where ranges are smaller, and 2° pixels may be wider than the available habitat, for example, the width of Java or the Malayan Peninsula. Lion population density is lower in deserts and semi-deserts compared to moist savannahs, whilst home-range size is smaller in moist savannah than desert areas (Celesia et al., 2010). Lion population density is influenced by multiple factors, and is related positively to herbivore biomass, annual mean temperature, annual mean temperature and soil nutrients (Celesia et al., 2010). Therefore it would be preferable to model distributions using the finest resolution possible, whilst still allowing for efficient computing. Conversely, care must be taken in the interpretation of high-resolution models, which may provide a false impression of accuracy on distributions based on palaeo-climatic models which rely upon the interpolation of sparse data.

Data processing and evaluation

Specific modelling frameworks have their own requirements in terms of data and best practices in setting parameters, which are further elaborated upon in *Chapters 4 and 5*, however certain considerations are widely applicable. The use of spatially autocorrelated occurrence data can inflate the area under the receiver-operator curve (AUC) derived from the modelling process (Veloz, 2009), and thus compromise a key measure of overall model performance. In niche modelling techniques, it is good practice to remove highly correlated variables from the modelling process to reduce over-parameterisation and loss of predictive power (Buermann et al., 2008; Garcia-Porta et al., 2012). When two or more variables are highly correlated with each other, it is preferable to include variables that are deemed relevant to the survival of the species in question. Spatial autocorrelation of locality data may arise for ecological reasons, such as environmental factors that limit the mobility of an organism, or behavioural factors that cause the spatial aggregation of species within

landscapes (Dormann et al., 2007). Different modelling techniques can produce highly variable results (Pearson et al., 2006) and therefore reliance on one technique may limit the usefulness of a model. Modelling distributions based on a single model could potentially inflate the users' confidence in the model output if the variability between different modelling techniques is not accounted for. In light of these concerns a sensitivity analysis is conducted within the Supplementary Information of *Chapter 3*, which informs the models presented in both *Chapters 3* and *4*.

The creation of habitat suitability maps for past climatic conditions creates testable scenarios on which the likelihood of a species' presence for a given time period can be assessed. Ideally, species distribution models are evaluated with independent observation data. However, with cryptic and mobile species, such as big cats, these data are often not available. Instead, cross-validation can be performed using replicates to assess each model. This technique uses all of the data in validating the model. Cross-validation is the preferred approach for evaluating presence-only niche models because, as climate variables used are commonly strongly correlated with each other, goodness-of-fit statistics are usually highly inflated (Hijmans, 2012). A cross-validation approach provides a measure of predictive power rather than significance, which is generally more relevant in niche models in which the objective is often prediction. Beyond the statistical evaluation of models created, validation can come from their correspondence with genetic and morphological studies, palaeontological records, historical records, evidence from rock art and alternative climatic proxy data, all of which can give insight into the validity of created models.

The methods of *Chapter 3* assess the efficacy of a Maximum Entropy approach to model the range of the tiger. The sensitivity analysis conducted therein provides support for the methods chosen and acts as a foundation for the methods used in *Chapter 4*. Whilst Maximum Entropy modelling is also employed within *Chapter 4*, it is built upon using a novel descriptive framework based around the creation and use of Global Environmental Strata and Zones (Metzger et al., 2013; Soteriades et al., 2017) on palaeo-climatic conditions.

Morphological Analysis

This thesis is concerned with the recent evolutionary histories of the lion and tiger at timescales spanning tens of thousands of years, however due to known variation in morphology due to life history (Smuts et al., 1978; Currey, 2003), morphology must also be considered in relation to much shorter timescales. The available data and it's applicability for morphological analysis is discussed within this section. Tigers display a relatively high level of skull diversity when compared to other widely distributed big cats (Mazák, 2010) and therefore the tiger, and similarly sized lion, are good candidate species to explore geographical variation in relation to the environmental conditions of different populations.

Data

The morphological dataset available in this study consists of 77 linear measurements from 893 lion and tiger skulls from primarily European museum collections, provided by Nobuyuki Yamaguchi, and further supplemented by myself, following the measurements presented in Barnett et al., (2008). Both wild and captive specimens are represented within the dataset which provides the opportunity to test differences in the life histories of individuals given the different lifestyles and feeding regimes dictated by captivity status (O'Regan & Kitchener, 2005), which is the focus of *Chapter 5*. There are however, collection biases which place limits on the analysis which can be performed and conclusions ascertained. The recent anthropogenic extirpation of several populations of the tiger and lion has limited the availability of these specimens for collection. The Balinese, South China and Caspian tiger, and North African and Asian populations of the lion are particularly underrepresented, yet may represent distinct taxonomic units (Bertola et al., 2016; Liu et al., 2018) and therefore be of interest to the analyses presented. Similarly there are biases in the geographic origins of captive specimens of the lion and tiger. The Amur and Sumatran tiger are particularly well represented in both captivity and the wild, yet the other populations, such as the Bengal tiger, are only well represented in the wild. These biases are further discussed in *Chapters 5+6*.

The use of linear morphometrics over more recent geometric techniques (e.g. Langerhans et al., 2003; Mazák et al., 2011; Hartstone-Rose et al., 2014), has enabled the assembly of a vast

dataset in terms of measurements and specimens, due to the minimal equipment required for data collection and the speed which measurements can be collected. Simple linear measurements also make it simpler to test the robustness of particular measurements, by using repeat measurements from multiple researches of the same specimens (*see Chapter 4*).

Analysis of the cranial skeleton is commonly used to assess geographic patterns and evolutionary history in mammals (e.g. Gay & Best, 1996; Viguier, 2002; Cardini, 2003; Christiansen, 2008; Mazák, 2010; Mazák et al., 2011), however this is only one aspect of morphology. Differences in the axial skeleton (Randau et al., 2016) and pelage (Yamaguchi et al., 2004; Wilting et al., 2015) of individuals may also provide a useful measure of variation across the range of both the lion and tiger. However, skull data is appropriate for assessing the consistency of variation within and between populations due to the large scale availability of skull data in museum collections, when compared to postcranial skeletons and furs.

Missing data

Whilst it is preferable to work on complete data, as many ordination techniques such as Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) cannot handle missing values, incomplete data is common in morphometrics (Clavel et al., 2014). Fragile areas of the skull may be more often damaged in comparison to stronger or better protected areas (Couette & White, 2010). Missing data in the morphological dataset presented here occurs due to specimen incompleteness from damage (accidental or from intrusive methods of previous investigation), from missing parts (e.g. teeth or mandibles), and from measurements simply missed by the researcher. Working on complete cases by removing missing data by specimen or by variable can lead to drastic reductions in the available data (Rhode & Arriaza, 2006). For the dataset used in *Chapters 5+6*, 10% of missing data affects 65% of specimens which is an unacceptable loss of data for use in further analysis.

Instead of removing variables with missing data, missing values are substituted with plausible values through imputation. Single imputation procedures do not account for imputation uncertainty (Azur et al., 2012) and tend to erase inter-specimen relations due to data homogenisation (Clavel et al., 2014) which is undesirable in the context of the analysis

presented in *Chapters 5+6*. Multiple imputations are used instead, which fill in missing values multiple times, thereby creating multiple datasets of complete values. The creation of multiple datasets incorporates the uncertainty in the procedure into the analysis. Multiple imputation by chained equations (MICE) is a common method of addressing missing data and working under the Missing At Random (MAR) assumption (Azur et al., 2012; Baur et al., 2014; Clavel et al., 2014). To avoid bias in the analysis model, the imputation model must include all variables to be used in the analysis model (Moons *et al*., 2006). However, it is not of practical importance whether all variables in the imputation model are included in the analysis model (White *et al*., 2011). Therefore data of both lion and tiger specimens are imputed together.

A potential concern of the MICE algorithm is that it assumes that missing data is missing at random (the probability that a value is missing depends only on the observed values and does not depend on unobserved values (Schafer & Graham, 2002). This assumption is likely to be violated - the likelihood of any given mandible measurement being missing will be dependent on whether other mandible measurements are missing, given that the whole mandible could well be absent from the measurements through being lost or damaged. Implementing MICE when the data is not missing at random could lead to biased estimates (Azur et al., 2012). Despite this, multiple imputation techniques have been shown to be robust against such assumption violations (Clavel et al., 2014). Including the large number of predictors that are available to this study in MICE makes the assumption of Missing at Random (MAR) more plausible, thereby reducing the need to make adjustments for Missing not at Random (MNAR) mechanisms (Schafer, 1997; White *et al*., 2011).

Data analysis

The core technique used to establish variation in skull shape within the lion and tiger in *Chapters 3+4* is Principal Component Analysis (PCA). This is chosen in preference of the similar ordination technique of Linear Discriminant Analysis, which is also commonly used in morphometric studies (see Platz et al., 2011; Baur et al., 2014; Tomassini et al., 2014; Hernández-Romero et al., 2015), due to the high dimensional nature of our data, and small sample sizes when different groups are separated. High dimensional, low sample size (HDLSS) data is problematic with Linear Discriminant Analysis as it can result in data piling

and the overfitting of results (Marron et al., 2007; Qiao et al., 2008). In the context of this research, morphological differences between taxonomically uncertain and poorly represented populations of the lion and tiger could be over exaggerated using these techniques. Principal component analysis reduces the set of measurements into linearly uncorrelated variables. This technique does not require the prior grouping of data, and so provides a more natural assessment of variation.

Allometry in morphology refers to size related changes of morphological traits (Klingenberg, 2016), and within skulls this manifests as non-linear changes to particular structures with size. Size is therefore accounted for by scaling the measurements before conducting PCA's, and by calculating the overall isometric size of a skull in accordance with (Baur & Leuenberger, 2011). Therefore, the PCAs conducted are independent of overall size, yet size related shape changes can be assessed by comparing the principal components with overall skull size. This process is described in more detail within *Chapter 5*.

Georeferencing of wild specimens

Museum specimens collected through the late 19th to early 21st century typically have some form of textual description recording the location the animal was collected from, be this in captivity, or in the wild. This recorded data may be vague, relating solely to the putative subspecies of the individual from which a broad geographic origin can be interpreted, or may be of a much higher resolution, to within tens of kilometres. Georeferenced locality descriptions from this morphological dataset have been employed within the biogeographical modelling described in *Chapters 3+4*, when location accuracy is below 50km. The georeferencing of these textual descriptions, which is expanded upon in *Chapter 6*, enables the explicit geographical analysis of skull morphology, which further connects the two strands of enquiry of this theses by a shared geographical association. The biogeographical modelling of *Chapters 3* and *4* and the analysis of skull plasticity in *Chapter 5*, provide vital context to the nature of morphological change explored in *Chapter 6*.

References

Alvarado-Serrano D.F. & Knowles L.L. (2014) Ecological niche models in phylogeographic studies: applications, advances and precautions. *Molecular ecology resources*, **14**, 233–48.

Araújo M.B. & Guisan A. (2006) Five (or so) challenges for species distribution modelling. *Journal of Biogeography*, **33**, 1677–1688.

Azur M.J., Stuart E.A., Frangakis C., & Leaf P.J. (2012) NIH Public Access. **20**, 40–49.

Barnett R., Yamaguchi N., Shapiro B., & Sabin R. (2008) Ancient DNA analysis indicates the first English lions originated from North Africa. *Contributions to Zoology*, **77**, 7–16.

Baur H., Kranz-Baltensperger Y., Cruaud A., Rasplus J.Y., Timokhov A. V., & Gokhman V.E. (2014) Morphometric analysis and taxonomic revision of Anisopteromalus Ruschka (Hymenoptera: Chalcidoidea: Pteromalidae) - an integrative approach. *Systematic Entomology*, **39**, 691–709.

Baur H. & Leuenberger C. (2011) Analysis of ratios in multivariate morphometry. *Systematic Biology*, **60**, 813–825.

Bertola L.D., Jongbloed H., van der Gaag K.J., de Knijff P., Yamaguchi N., Hooghiemstra H., Bauer H., Henschel P., White P.A., Driscoll C.A., Tende T., Ottosson U., Saidu Y., Vrieling K., & de Iongh H.H. (2016) Phylogeographic Patterns in Africa and High Resolution Delineation of Genetic Clades in the Lion (Panthera leo). *Scientific Reports*, **6**, 1–11.

Buermann W., Saatchi S., Smith T.B., Zutta B.R., Chaves J.A., Milá B., & Graham C.H. (2008) Predicting species distributions across the Amazonian and Andean regions using remote sensing data. *Journal of Biogeography*, **35**, 1160–1176.

Cardini A. (2003) The geometry of the marmot (Rodentia: Sciuridae) mandible: Phylogeny and patterns of morphological evolution. *Systematic Biology*, **52**, 186–205.

Celesia G.G., Townsend Peterson a., Kerbis Peterhans J.C., & Gnoske T.P. (2010) Climate and landscape correlates of African lion (Panthera leo) demography. *African Journal of Ecology*, **48**, 58–71.

Christiansen P. (2008) On the distinctiveness of the Cape lion (Panthera leo melanochaita Smith, 1842), and a possible new specimen from the Zoological Museum, Copenhagen. *Mammalian Biology*, **73**, 58–65.

Clavel J., Merceron G., & Escarguel G. (2014) Missing data estimation in morphometrics:

How much is too much? *Systematic Biology*, **63**, 203–218.

Couette S. & White J. (2010) 3D geometric morphometrics and missing-data. Can extant taxa give clues for the analysis of fossil primates? *Comptes Rendus Palevol*, **9**, 423–433.

Currey J.D. (2003) The many adaptations of bone. *Journal of Biomechanics*, **36**, 1487–1495.

Dormann C.F., McPherson J.M., Araújo M.B., Bivand R., Bolliger J., Carl G., Davies R.G., Hirzel A., Jetz W., Daniel Kissling W., Kühn I., Ohlemüller R., Peres-Neto P.R., Reineking B., Schröder B., Schurr F.M., & Wilson R. (2007) Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. *Ecography*, **30**, 609–628.

Eloff F.C. (1973) Water use by the Kalahari Lion Panthera leo vernayi. *Koedoe*, **16**, 149–154.

Garcia-Porta J., Litvinchuk S.N., Crochet P.A., Romano A., Geniez P.H., Lo-Valvo M., Lymberakis P., & Carranza S. (2012) Molecular phylogenetics and historical biogeography of the west-palearctic common toads (Bufo bufo species complex). *Molecular phylogenetics and evolution*, **63**, 113–30.

Gay S.W. & Best T.L. (1996) Relationships betwen abiotic variables and geographic variation in skull of pumas (Puma concolor: Mammalia, Felidae) in North and South America. *Zoological Journal of Linnean Society*, **117**, 259–282.

Geffen E., Anderson M.J., & Wayne R.K. (2004) Climate and habitat barriers to dispersal in the highly mobile grey wolf. *Molecular ecology*, **13**, 2481–90.

Hartstone-Rose A., Selvey H., Villari J.R., Atwell M., & Schmidt T. (2014) The Three-Dimensional Morphological Effects of Captivity. *PLoS ONE*, **9**, e113437.

Hernández-Romero P.C., Guerrero J.A., & Valdespino C. (2015) Morphological variability of the cranium of Lontra longicaudis (Carnivora: Mustelidae): a morphometric and geographic analysis. *Zoological Studies*, **54**, 50.

Hijmans R.J. (2012) Cross-validation of species distribution models: removing spatial sorting bias and calibration with a null model. *Ecology*, **93**, 679–88.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., & Jarvis A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Kanagaraj R., Wiegand T., Kramer-Schadt S., Anwar M., & Goyal S.P. (2011) Assessing habitat suitability for tiger in the fragmented Terai Arc Landscape of India and Nepal. *Ecography*, **34**, 970–981.

Khorozyan I. (2014) Morphological variation and sexual dimorphism of the common leopard (Panthera pardus) in the Middle East and their implications for species taxonomy and

conservation. *Mammalian Biology - Zeitschrift für Säugetierkunde*, **79**, 398–405.

Kitchener A.C. & Dugmore A.J. (2000) Biogeographical change in the tiger , Panthera tigris. *Animal Conservation*, **3**, 113–124.

Klingenberg C.P. (2016) Size, shape, and form: concepts of allometry in geometric morphometrics. *Development Genes and Evolution*, **226**, 113–137.

Kozak K.H., Graham C.H., & Wiens J.J. (2008) Integrating GIS-based environmental data into evolutionary biology. *Trends in Ecology & Evolution*, **23**, 141–8.

Langerhans R.B., Layman C.A., Langerhans A.K., & Dewitt T.J. (2003) Habitat-associated morphological divergence in two Neotropical fish species. *Biological Journal of the Linnean Society*, **80**, 689–698.

Liu Y.-C., Sun X., Driscoll C., Miquelle D.G., Xu X., Martelli P., Uphyrkina O., Smith J.L.D., O'Brien S.J., & Luo S.-J. (2018) Genome-Wide Evolutionary Analysis of Natural History and Adaptation in the World's Tigers. *Current Biology*, **28**, 3840–3849.e6.

Lorenzen E.D., Nogués-Bravo D., Orlando L., Weinstock J., Binladen J., Marske K. a, Ugan A., Borregaard M.K., Gilbert M.T.P., Nielsen R., Ho S.Y.W., Goebel T., Graf K.E., Byers D., Stenderup J.T., Rasmussen M., Campos P.F., Leonard J. a, Koepfli K.-P., Froese D., Zazula G., Stafford T.W., Aaris-Sørensen K., Batra P., Haywood A.M., Singarayer J.S., Valdes P.J., Boeskorov G., Burns J. a, Davydov S.P., Haile J., Jenkins D.L., Kosintsev P., Kuznetsova T., Lai X., Martin L.D., McDonald H.G., Mol D., Meldgaard M., Munch K., Stephan E., Sablin M., Sommer R.S., Sipko T., Scott E., Suchard M. a, Tikhonov A., Willerslev R., Wayne R.K., Cooper A., Hofreiter M., Sher A., Shapiro B., Rahbek C., & Willerslev E. (2011) Species-specific responses of Late Quaternary megafauna to climate and humans. *Nature*, **479**, 359–64.

Marron J.S., Todd M.J., & Ahn J. (2007) Distance-Weighted Discrimination. *Journal of the American Statistical Association*, **102**, 1267–1271.

Martínez-Meyer E., Peterson A.T., & Hargrove W.W. (2004) Ecological niches as stable distributional constraints on mammal species , with implications for Pleistocene extinctions and climate change projections for biodiversity. *Global Ecology and Biogeography*, **13**, 305–314.

Mazák J.H. (2010) Craniometric variation in the tiger (Panthera tigris): Implications for patterns of diversity, taxonomy and conservation. *Mammalian Biology - Zeitschrift für Säugetierkunde*, **75**, 45–68.

Mazák J.H., Christiansen P., & Kitchener A.C. (2011) Oldest Known Pantherine Skull and

Evolution of the Tiger. *PloS one*, **6**, 1–11.

Metzger M.J., Bunce R.G.H., Jongman R.H.G., Sayre R., Trabucco A., & Zomer R. (2013) A high-resolution bioclimate map of the world: A unifying framework for global biodiversity research and monitoring. *Global Ecology and Biogeography*, **22**, 630–638.

Moons K.G.M., Donders R.A.R.T., Stijnen T., & Harrell F.E. (2006) Using the outcome for imputation of missing predictor values was preferred. *Journal of Clinical Epidemiology*, **59**, 1092–1101.

Mowat G. & Heard D.C. (2006) Major components of grizzly bear diet across North America. *Canadian Journal of Zoology*, **84**, 473–489.

Nowell K. & Jackson P. (1996) *The Wildcats: A Status Survey and Conservation Action Plan.* IUCN, Gland, Switzerland.

O'Regan H.J. & Kitchener A.C. (2005) The effects of captivity on the morphology of captive, domesticated and feral mammals. *Mammal Review*, **35**, 215–230.

Pearson R.G., Dawson T.P., & Liu C. (2004) Modelling species distributions in Britain : a hierarchical integration of climate and land-cover data. **3**, 285–298.

Pearson R.G., Raxworthy C.J., Nakamura M., & Townsend Peterson A. (2006) Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography*, **34**, 102–117.

Phillips S.J., Anderson R.P., & Schapire R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.

Platz S., Hertwig S.T., Jetschke G., Krüger M., & Fischer M.S. (2011) Comparative morphometric study of the Slovakian wildcat population (Felis silvestris silvestris): Evidence for a low rate of introgression? *Mammalian Biology*, **76**, 222–233.

Qiao Z., Zhou L., & Huang J.Z. (2008) Effective linear discriminant analysis for high dimensional , low sample size data. II,

Randau M., Goswami A., Hutchinson J.R., Cuff A.R., & Pierce S.E. (2016) Cryptic complexity in felid vertebral evolution: shape differentiation and allometry of the axial skeleton. *Zoological Journal of the Linnean Society*, .

Raxworthy C.J., Ingram C.M., Rabibisoa N., & Pearson R.G. (2007) Applications of ecological niche modeling for species delimitation: a review and empirical evaluation using day geckos (Phelsuma) from Madagascar. *Systematic biology*, **56**, 907–23.

Rhode M.P. & Arriaza B.T. (2006) Influence of cranial deformation on facial morphology among prehistoric South Central Andean populations. *American Journal of Physical Anthropology*, **130**, 462–470.

Schafer J.L. (1997) *Analysis of Incomplete Multivariate Data.* Chapman & Hall, London.

Schafer J.L. & Graham J.W. (2002) Missing data: our view of the state of the art. *Psychological Methods*, **7**, 147–177.

Smuts G.L., Anderson J.L., & Austin J.C. (1978) Age determination of the African lion (Panthera leo). *Journal of Zoology*, **185**, 115–146.

Soteriades A.D., Murray-Rust D., Trabucco A., & Metzger M.J. (2017) Understanding global climate change scenarios through bioclimate stratification. *Environmental Research Letters*, **12**, 1–10.

Stevens G.C. (1989) The latitudinal gradient in geographical range: How so many species coexist in the tropics. *The American Naturalist*, **133**, 240–256.

Sunquist M.E. (1981) The Social Organization of Tigers (Panthera Tigris) in Royal Chitawan National Park, Nepal. *Smithsonian Contributions to Zoology*, **336**, 1–98.

Tomassini A., Colangelo P., Agnelli P., Jones G., & Russo D. (2014) Cranial size has increased over 133 years in a common bat, Pipistrellus kuhlii: A response to changing climate or urbanization? *Journal of Biogeography*, **41**, 944–953.

Tseng Z.J., Wang X., Slater G.J., Takeuchi G.T., Li Q., Liu J., & Xie G. (2014) Himalayan fossils of the oldest known pantherine establish ancient origin of big cats. *Proc Biol Sci*, **281**, 20132686.

Varela S., Lobo J.M., Rodríguez J., & Batra P. (2010) Were the Late Pleistocene climatic changes responsible for the disappearance of the European spotted hyena populations? Hindcasting a species geographic distribution across time. *Quaternary Science Reviews*, **29**, 2027–2035.

Veloz S.D. (2009) Spatially autocorrelated sampling falsely inflates measures of accuracy for presence-only niche models. *Journal of Biogeography*, **36**, 2290–2299.

Viguier B. (2002) Is the morphological disparity of lemur skulls (Primates) controlled by phylogeny and/or environmental constraints? *Biological Journal of the Linnean Society*, 577–590.

Waltari E., Hijmans R.J., Peterson A.T., Nyári A.S., Perkins S.L., & Guralnick R.P. (2007) Locating pleistocene refugia: comparing phylogeographic and ecological niche model predictions. *PloS one*, **2**, 1–11.

White I.R., Royston P., & Wood A.M. (2011) Multiple imputation using chained equations: Issues and guidance for practice. *Statistics in Medicine*, **30**, 377–399.

Wilting A., Courtiol A., Christiansen P., Niedballa J., Scharf A.K., Orlando L., Balkenhol N., Hofer H., Kramer-Schadt S., Fickel J., & Kitchener A.C. (2015) Planning tiger recovery:

Understanding intraspecific variation for effective conservation. *Science Advances*, **1**, 1–13.

Yamaguchi N., Cooper A., Werdelin L., & Macdonald D.W. (2004) Evolution of the mane and group-living in the lion (Panthera leo): A review. *Journal of Zoology*, **263**, 329–342.

Zomer R.J., Bossio D.A., Trabucco A., Yuanjie L., Gupta D.C., & Singh V.P. (2007) Trees and Water: Smallholder agroforestry on irrigated lands in Northern India. Columbo, Sri Lanka: International Water Management Institute. .

Zomer R.J., Trabucco A., Bossio D.A., & Verchot L. V. (2008) Climate change mitigation: A spatial analysis of global land suitability for clean development mechanism afforestation and reforestation. *Agriculture, Ecosystems and Environment*, **126**, 67–80.

Chapter 3: Predicted Pleistocene-Holocene range shifts of the tiger

Preface

This chapter is the first of two modelling chapters, and assesses the degree of natural connectivity between current, and Late Pleistocene distributions of the tiger. The modelling approach assessed and conducted here is utilised, and built upon in Chapter 4 which concentrates on the range of the lion. This chapter has been published in *Diversity and Distributions* (Cooper et al., 2016) which is included as *Appendix 1* of the thesis. The author contributions are detailed at the start of the Supplementary Information for this chapter.

Abstract

Aim In this chapter the potential range shifts of tiger (*Panthera tigris*) populations are modelled over the Late Pleistocene and Holocene, in order to provide new insights into the evolutionary history and interconnectivity between populations of this endangered species.

Location Asia

Methods An ecological niche approach and applied a maximum entropy (MaxEnt) framework is used to model potential distributions of tigers. Bioclimatic conditions for the present day and mid-Holocene, and for the Last Glacial Maximum (LGM), were used to represent interglacial and glacial conditions of the Late Pleistocene respectively.

Results The results presented here show that the maximum potential tiger range during modern climates (without human impacts) would be continuous from the Indian subcontinent to northeast Siberia. During the LGM distributions are predicted to have contracted to southern China, India and Southeast Asia, and remained largely contiguous. A potential distribution gap between Peninsular Malaya and Sumatra could have effectively separated tigers on the Sunda Islands from those in continental Asia during interglacials.

Main Conclusions The continuous modelled distribution of tigers in mainland Asia supports the idea of mainly unimpeded gene flow between all populations throughout the Late Pleistocene and Holocene. Thus the data support a pragmatic approach to tiger conservation management, especially of mainland populations, as it is likely that only recent anthropogenic changes caused separation of these populations. In contrast, Sunda tigers are likely to have separated and differentiated following the Last Glacial Maximum and thus warrant separate management.

Keywords Ecological Niche Model, Evolutionary History, Maximum Entropy, Panthera tigris, Pleistocene Distribution, Toba

Introduction

Tigers are endangered and occupy only a small fraction of their historical range (Walston *et al*., 2010). The changing isolation or interconnectivity of tiger populations has wide-ranging implications for both *in-situ* and *ex-situ* conservation efforts, as conservationists currently aim to preserve what are assumed to be genetically distinct populations whilst attempting to strengthen the numbers of captive and wild tiger populations. This chapter presents a new, geographically-based assessment of changes in tiger distribution during the late Pleistocene and Holocene, in order to understand the degree of natural connectivity between separate tiger populations and thus inform current conservation efforts.

With a large sub-continental-scale geographical range, it is not surprising that tigers display morphological variation in response to regional differences in climate and habitat. This differentiation is reflected in the recognition of up to nine subspecies of tiger (Goodrich *et al*., 2015, but see Wilting *et al*. 2015) [*Figure 1(a)*], and these are the focus of current conservation efforts. However, the scientific integrity of so many subspecies has been questioned (e.g. Herrington, 1987; Kitchener, 1999; Kitchener & Yamaguchi, 2010; Wilting *et al*., 2015). It is possible these apparent patterns of variation are the result of genetic drift due to increasing recent fragmentation of tiger populations (Luo *et al*., 2004; Mondol *et al*., 2013), but lack conservation and evolutionary significance (Wilting *et al*. 2015). Although the fossil record of the tiger stretches back more than 2.5 million years (Mazák et al., 2011), studies of mitochondrial DNA have estimated that the most recent common ancestor for today's tiger populations existed 72-108ka (Luo e*t al*., 2004, Wilting *et al*. 2015). As a result, environmental changes in the late Pleistocene and early Holocene are most relevant when trying to understand differentiation of today's local tiger populations. For example, Sunquist (1981) suggested that the initial adaptive radiation of modern tigers may have occurred during the Pleistocene glaciations when Southeast Asian climates were drier (Whitmore, 1984), sea levels were lower, the Sunda Islands were linked by land to the Asian mainland, and insular and mainland populations could mix. Subsequent sea-level rises isolated tigers of the Sunda Islands from mainland tigers and each other, possibly leading to local differentiation. In addition to range changes driven by glacial cycles, the super-eruption of Toba ca.73ka in northern Sumatra [*Figure 1(c)*] may have played a key role in the evolutionary history of tigers through extensive habitat loss. This eruption produced around 2500-3000km^3 of dense rock-equivalent pyroclastic ejecta (Rose & Chesner, 1987) with

associated Younger Toba tuff (YTT) deposits found in cm–scale thicknesses across the Indian subcontinent (Acharyya & Basu, 1993; Shane et al., 1995), and at a thickness of more than four metres close to the Toba caldera (Oppenheimer, 2002). The outflow of m-scale deposits of YTT could have resulted in the ignition of vegetation across 30,000km^2 around the caldera (Oppenheimer, 2002).

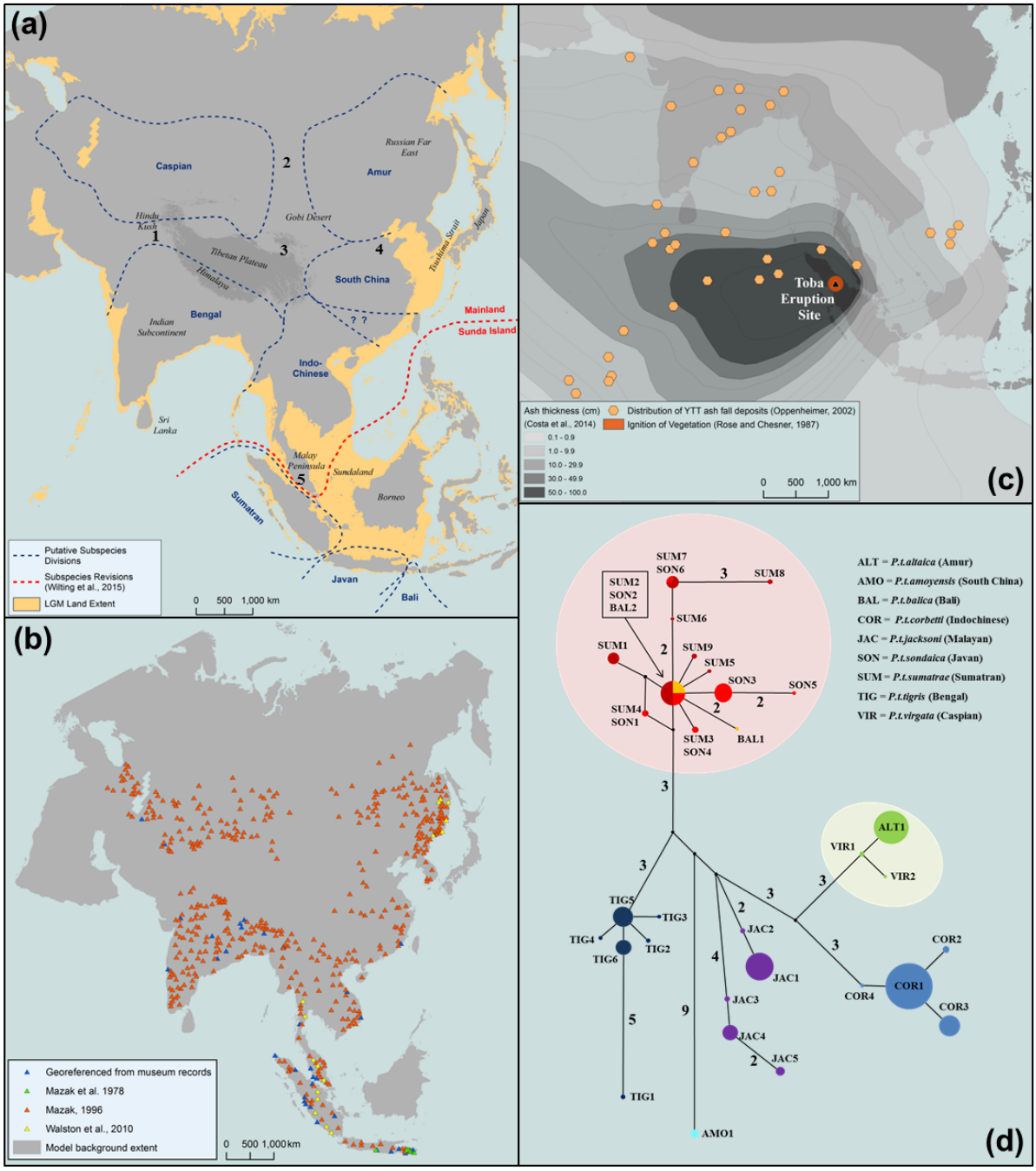


Figure 1: (a): Established subspecies divisions (Mazak, 1996), and revised divisions (Wilting *et al.*, 2015). Potential corridors for tiger dispersal between populations are (1) Southern/Himalayan Corridor, (2) Northern Corridor, (3) Gansu/Silk Road Corridor (Driscoll *et al.*, 2009). (4) Huanghe river catchment and site of human agricultural civilisation ca.8ka, (5) Additional recognition of a Malayan tiger (*P.t.jacksoni*) (Luo *et al.*, 2004). **(b)**: Tiger locality data, coloured by data source, with the background extent used for modelling. **(c):** Effects of the 73ka Toba super-eruption. The initial eruption effects, cooler temperatures and drought induced by the ejecta, followed by rapidly decreasing tree cover in the decades following the Toba eruption, might have been responsible for genetic bottlenecks in Southeast Asia (Robock *et al.*, 2009; Williams *et al.*, 2009; Wilting *et al.*, 2011, 2012). **(d)**: Haplotype network of nine putative subspecies, based upon 14 mitochondrial primers (Wilting *et al.*, 2015) – Circle size is proportional to haplotype frequency; lines represent a single mutational step, or multiple steps as indicated by numbers.

The evolutionary history of modern tiger populations has been assessed phylogenetically based on geographically referenced specimens (or at least specimens from known putative subspecies) and the analysis of mitochondrial DNA (Cracraft et al., 1998; Luo et al., 2004; Driscoll et al., 2009; Wilting et al., 2015; Xue et al., 2015), and/or nuclear microsatellite genotypes (Luo *et al*., 2004). A haplotype network was constructed by Wilting *et al*., (2015) [*Figure 1(d)*]. Phylogenetic approaches to understanding tiger populations are constrained because they are based on limited sampling of already fragmented populations (Luo *et al.,* 2004*)*. Additionally, current phylogeographical studies e.g. Luo *et al*., (2004), Driscoll *et al*., (2009) have only a limited spatial component (Waltari et al., 2007; Kozak et al., 2008). To help understand phylogeographical patterns seen today, there is a need for a geographically explicit understanding of the expansion and contraction of tiger ranges during glacial/interglacial cycles.

The understanding of the evolutionary history of tigers is of great importance for the successful conservation of this highly threatened species. Recognising too few differentiated populations could see the loss of important evolutionary diversity, but recognising too many would lead to a waste of resources and may compromise conservation efforts because some remaining populations are too small to survive (Bay et al., 2014). In contrast to some molecular studies which support high differentiation among mainland tiger populations (Luo *et al*., 2014, but see Wilting *et al*., 2015), Kitchener & Dugmore (2000) used a biogeographical approach and their results showed a considerable contiguity between mainland tiger populations. However 16 years have elapsed since that research was undertaken, and there is scope for radical improvement using new and improved modelling techniques and data. Here the global distribution of the tiger is examined using a maximum entropy (MaxEnt) niche modelling approach to reconstruct the current range of the tiger based on bioclimatic variables, assuming no human impacts. Furthermore the global ranges at the LGM and mid-Holocene are modelled to represent the maximum range of bioclimatic variation to impact tiger distribution, and to assess potential differentiation by isolation since modern populations originated ca.100ka.

Methods

Tigers need access to water, and they require dense vegetation cover of sufficient area to support large ungulates and to hunt their prey successfully (Nowell & Jackson, 1996). Therefore, the modelling approach used here assumes that tigers are generalists, operating within certain kinds of vegetation cover, and prey size and abundances that are dictated by climate. Climate is a key factor in defining ecological niches and the geographical distribution of species at continental scales (Geffen et al., 2004; Varela et al., 2010), and has been used to model megafaunal ranges (Varela et al., 2010; Lorenzen et al., 2011). Niche models, comparing recent and fossil locality records, have shown that mammal species have tracked consistent climate profiles since the LGM (Martínez-Meyer et al., 2004) and therefore climate change may be used to infer differences in mammal range as it is unlikely that a generalist, adaptable carnivore such as the tiger has shifted climatic niche through the Late Pleistocene.

Tiger locality records were taken from Mazák (1996) (448 locations), Walston *et al*. (2010) (16 locations), Mazák *et al*. (1978) (7 locations), and 40 localities georeferenced from European museum specimens [*Figure 1(b)*]. The records cover the geographical extent of the known distribution of tigers in modern times, and likely represent the potential climatic niche of the tiger, but it cannot be discounted that human extirpation of the tiger has reduced the potential niche space represented by these records. Nine contemporary WorldClim bioclimatic variables (Hijmans *et al*., 2005), in combination with the tiger localities, were used to produce a global habitat suitability model for the tiger using MaxEnt.

Of *presence only* modelling techniques, MaxEnt models have been shown to perform as well or better than other existing approaches (Elith et al., 2006; Hernandez et al., 2006; Phillips et al., 2006). Hernandez *et al*., (2006) found that MaxEnt performed well regardless of the number of species records or the geographical extent of records, compared to Mahalanobis Typicalities and Random Forests methods. MaxEnt software (Phillips et al., 2006) was used for modelling. In addition to MaxEnt a Boosted Regression Tree (BRT) model was created for comparison (*see Appendix S1 in Supplementary Information*). Models were created using WorldClim variables at a 2.5-arc-minute resolution (<5km), which is between one and two orders of magnitude less than the scale of individual tiger ranges. This resolution was chosen

to show potential habitat suitability through narrow geographical corridors such as through valleys or mainland connections to peninsulas which may affect gene flow between tiger populations. Elevation was not included as a separate independent variable because it is accounted for in the bioclimatic datasets. In contrast to Kitchener & Dugmore (2000), vegetation data, such as Biome 4 (Kaplan *et al*., 2003), have not been included in the model, because they can decrease performance in predicting presences compared to models based only on climatic variables (Martínez-Meyer et al., 2004). A comparison with Biome 4 data was used as a post-modelling measure of validity, by checking that modelled high habitat suitability does not occur in highly unfavourable habitats (Desert and Tundra), as they are unequivocally not recognised as tiger habitat (Nowell & Jackson, 1996).

Highly correlated variables were removed from the modelling process to reduce over-parameterisation and loss of predictive power (Buermann *et al*., 2008; Garcia-Porta *et al*., 2012). The removal of highly correlated variables ($r \geq 0.8$), calculated using ArcGIS Band Collection Statistics (ArcMap v10.1), from the 2.5-arc-minute WorldClim bioclimatic dataset reduced the number of variables from 19 to nine (annual mean temperature, mean diurnal range, isothermality, mean temperature of the wettest quarter, precipitation seasonality, precipitation of the wettest quarter, precipitation of the driest quarter, precipitation of the warmest quarter, precipitation of the coldest quarter). Where one or more variables were highly correlated, the one deemed most important was selected using indicators, such as performance in a Jackknife test (Pearson et al., 2006).

MaxEnt models were run under settings so as to fit a poisson point process model – "noremoveduplicatepresencerecords" and "noaddsamplestobackground", with final projections displaying MaxEnt raw output (Renner *et al*., 2015). MaxEnt models were evaluated under a range of settings to assess their effects on model performance - see Appendix S1 for a full analysis of parameter selection. Final MaxEnt models were run using a regularization multiplier (RM) of 2, and 100,000 background points. Ideally, models would be evaluated with independent observation data; however, these data are not available. Instead, cross-validation was performed using replicates in MaxEnt to assess each model. Ten runs of each model were performed and the mean area under the receiver operating characteristic curve (AUC) was used as a measure of overall performance (Phillips & Dudík, 2008). Additionally, spatially independent cross validation was performed using the checkerboard2 method implemented via the ENMeval package (Muscarella *et al*., 2014) in R

(R Core Team, 2015)] to reduce the potential effects of spatially autocorrelated localities, which may inflate the AUC (Veloz, 2009). Checkerboard2 was implemented with coarse grids 200 and 400 times the resolution of the bioclimatic variables (~450km^2 and ~900km^2 at the equator respectively) to ensure considerable geographic separation of training and testing localities. Models were run over a geographical extent covering the known and potential distribution of the tiger (82°N to 10°S, 20°W to 144°E). Africa and islands not connected to continental Asia during glacial conditions, such as those east of the Wallace Line, were removed from the extent before modelling.

Following assessment, the model was fitted using all localities, and projected to the LGM at 21ka and mid-Holocene at 6ka, using WorldClim data derived from the MIROC-ESM, CCSM-4 and MPI-ESM-P coupled general circulation models (GCM's) (Hijmans *et al*., 2005) based on CMIP5 (Taylor et al., 2012) data. Model clamping was implemented to restrict variables to the range of values encountered during training. An ensemble projection was created for the Mid-Holocene and LGM based upon the mean raw value of the projected models from the three coupled GCM datasets for each time period. The individual projections and range of the projection values can be viewed in Appendix S1. The present/mid-Holocene and LGM climates provide bounding values of the climatic fluctuations of the Pleistocene, and thus likely represent environmental changes that influenced tiger distribution and potential range shifts. Whilst the present and mid-Holocene both represent interglacial climatic conditions, with both periods exhibiting similar global annual mean temperature and precipitation, the mid-Holocene is associated with an enhanced seasonal cycle in the Northern Hemisphere, and wetter conditions within eastern Asia (Braconnot et al., 2007; Tao et al., 2010), which may have affected tiger distributions.

Georeferenced fossil records of the tiger from the Late Pleistocene (Kitchener & Yamaguchi, 2010) are presented on the relevant glacial/interglacial habitat suitability map based on their estimated age [*Figure 2(a),(b)*]. The Late Pleistocene distribution of the ancestors of the modern lion (*Panthera leo*) and of the Eurasian cave lion (*Panthera leo spelaea*) (Barnett *et al*., 2009) have been superimposed onto modelled tiger distributions [*Figure 3(6)*], because where open habitat is dominant, it is expected that the group-living modern lion, and likely group-living Eurasian cave lion (Yamaguchi et al., 2004) would out-compete tigers and prevent their dispersal. Estimates of ashfall from the very large scale volcanic eruption of Toba ca.73ka (Costa et al., 2014) have also been added post-modelling [*Figure 3(1)*] to

assess their potential impacts on tiger distributions. The minimum values of the raw output between present day, mid-Holocene and LGM projections are presented in *Figure 3[~73ka(+1k)]*, to represent a rapid shift from interglacial to glacial conditions during the 1000 years following the Toba eruption (Rampino & Self, 1992; Harris, 2008).

Results

The tiger distribution model run under final parameters with cross-validation produced a mean AUC of 0.843, representing the probability of a randomly chosen presence locality being ranked above a random background point (Phillips et al., 2006). The same parameters, but run with spatially independent cross-validation, gave a mean AUC of 0.780. Under a point-process framework, the MaxEnt raw projection is viewed as the intensity of potential locality reportings within a given area (Renner *et al*., 2015), which for the projected models can be interpreted in terms of habitat suitability. Model sensitivity is further discussed in Appendix S1 - conclusions regarding tiger distributional changes from the chosen MaxEnt model are consistent with MaxEnt models created under a range of parameters, and with a simply implemented BRT model.

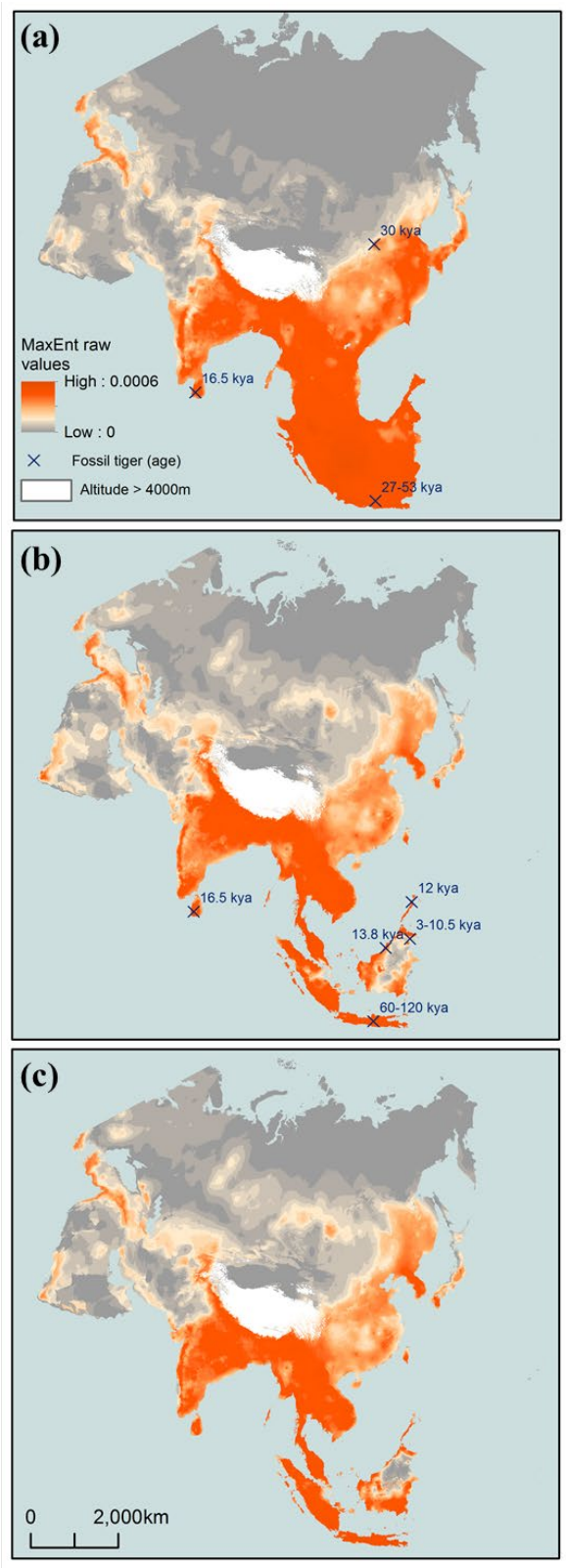


Figure 2: Raw MaxEnt output displaying modelled tiger habitat suitability based on nine bioclimatic variables, projected using an Asia Lambert Conformal Conic Projection, for the LGM **(a)**, mid-Holocene **(b)** and present day **(c)**. Elevations > 4000m (highlighted in white) have been included as a possible barrier to dispersal. Fossil tiger localities are laid over glacial **(a)** and interglacial **(b)** projections in accordance with the fossil age (note 16.5 kya from Sri Lanka is included in both scenarios due to the transitionary time period following the LGM.

The fossil record of tigers corresponds well with predicted habitat suitability maps for glacial/interglacial conditions [*Figure 1(a)+(b)*], although this evaluation method is limited by the scarcity of dated archaeological finds. A comparison of model projections with unfavourable vegetation provides support for past outputs, as strong modelled habitat suitability has not occurred in biomes deemed unfavourable (Appendix S1). Modelling the likely ranges for present-day climate [*Figure 2(a)*] indicates a potentially contiguous tiger distribution from southern India to the Amur region, and presence throughout the Malay Peninsula, Sumatra, Java and Bali. The present model suggests that tigers in the Caspian region existed within suboptimal habitat, and likely connected through corridors of favourable habitat to tiger populations in northern India, and the Amur region. The core areas of tiger habitat, defined as those suitable at present, mid-Holocene and during the LGM [*Figure 1(a)-(c)*]], exist within the Indian subcontinent and Southeast Asia..

Discussion

The present-day model corresponds well with the Habitat Topography Precipitation model of present tiger distribution (Kitchener & Dugmore, 2000) and historic tiger distributions during the Holocene (Dinerstein *et al*., 2006). However, the present model predicts low habitat suitability for central and northern Borneo, whereas past studies have modelled this as suitable habitat (Kitchener & Dugmore, 2000). One implication is that simple associations with forest cover, annual rainfall and altitude (Kitchener & Dugmore, 2000) do not reflect the true variability in habitat suitability. The modelled low habitat suitability on Borneo, compared to Sumatra, Java and Bali, shows that, whilst still forested, the environmental conditions of Borneo are different from those of the other Sunda Islands. Using the 'explain' tool in MaxEnt (see Appendix S1) it is apparent that precipitation seasonality is the driving factor behind modelled habitat suitability with lower precipitation seasonality causing lower suitability. Borneo exhibits lower prey densities than the other Sunda Islands, associated with seasonal precipitation events that affect flowering and fruiting (Wong et al., 2005), which have been linked to the absence of large carnivores in Borneo (Meijaard, 2004; Wong *et al*., 2005). Whilst tigers may have been present in Borneo up until recent times (Hooijer, 1963), decreasing habitat suitability from glacial to interglacial conditions, as suggested by Harrison, (1996) and supported by the modelling, may have contributed to an extirpation

driven by human impacts. It is likely that the inclusion of more complex environmental variables, which incorporate seasonal environmental variability, has highlighted important bioclimatic differences between the Sunda Islands that mirror significant ecological variation.

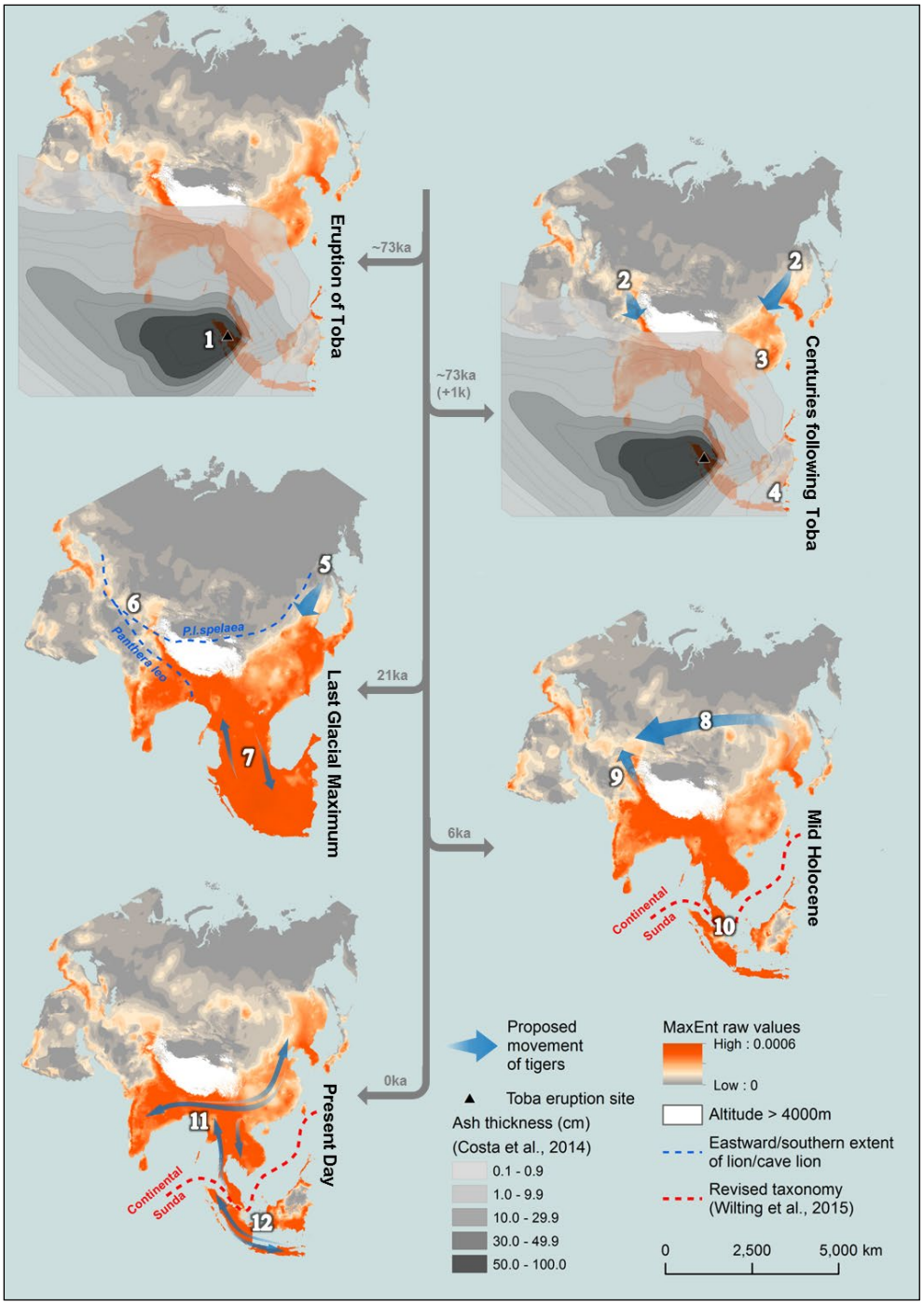


Figure 3: Proposed range shifts of the tiger since the ~73ka eruption of Toba (1). Immediate impacts (1), and millennial scale cooling, likely reduced the northern range (2), leading to a probable refugium (3), associated with the genetic bottleneck of the tiger. Suitable (but fragmented) habitats (4), were unlikely to have harboured the tiger during this time due to a single source of molecular variation. The northern range of the tiger would have been suppressed by unfavourable conditions at the LGM (5), and Caspian/western Indian marginal habitat may have been impacted by the distribution of the lion (6). LGM conditions likely allowed the free movement of the tiger between the Sunda Islands and the continent (7). Post LGM conditions saw the retreat of the lion range, which, along with more favourable habitat suitability would have allowed the colonisation of the Caspian region via a northern corridor (8), southern corridor (9) or concurrent corridors (8+9). Tigers would have been separated by rising sea levels following the LGM through the Strait of Malacca (10), leaving a contiguous population of Continental tigers (11), and Sunda tigers which were likely able to disperse between islands until modern times (12).

A key difference between the LGM model and those of previous biogeographical studies is the favourable habitat suitability modelled within the Indian subcontinent, which was predicted as poor habitat by Kitchener & Dugmore, (2000). Whilst habitat suitability is reduced in the northern range of the tiger, such as Siberia, it is expanded within the Sunda Shelf, and may have led to an increase in overall available tiger habitat during glacial conditions compared to interglacial conditions. Reduced habitat suitability found around the Caspian during the LGM [*Figure 2(a)*] diminishes the likelihood of a viable population during glacial conditions in this region.

The modelling reported here does not support the dispersal of tigers through the Silk Road/Gansu Corridor [*Figure 1(a)- route 3*] from China to central Asia and the Caspian (Driscoll *et al*., 2009). Even considering marginal environments suitable only for dispersal (*Figure 2*), this route is deemed improbable. More likely is a colonisation of the Amur region from northern China, followed by a post-LGM westward dispersal of tigers through a northern corridor [*Figure 1(a) – route 2, Figure 3(8), Figure 4(d-f)*]. Considering the potential rapidity of tiger dispersal - individuals have been known to travel up to 1000km (Kitchener, 1999), then it is probable that use of the northern corridor and gene flow through optimal interglacial and seasonal conditions would have resulted in low genetic variation between the Amur and Caspian tigers, and this is supported by molecular studies (Driscoll et al., 2009; Wilting et al., 2015)

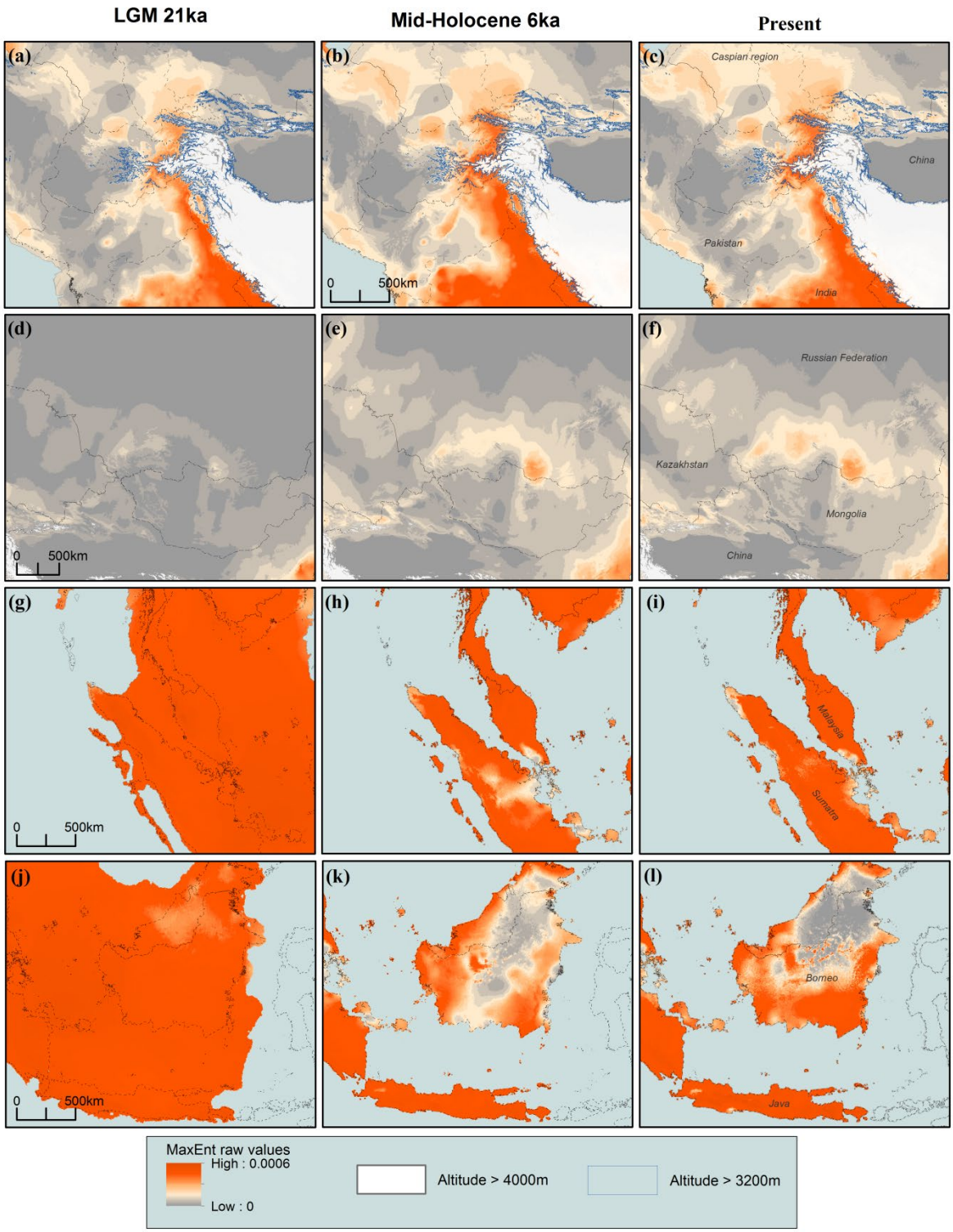


Figure 4: Detailed view of final model projections for the LGM, mid-Holocene and present conditions. In addition to elevations > 4000m (white areas), included as a possible barrier to tiger dispersal, elevations > 3200m are shown between India and the Caspian region [**(a) - (c)**] to delineate the regional treeline. Further image tiles show the proposed northern corridor between Amur and Caspian populations **[(d) - (f)**], the continental/Sundaland divide **[(g) - (i)**] and range shifts within the Sunda Islands [**(j) - (l)**].

Significant areas of suitable habitat are modelled along the southern route between the Indian subcontinent and the Caspian during interglacial conditions [*Figure 1(a) – route 1, Figure 3(9), Figure 4(a-c)*]. However, a lack of genetic affinity between Caspian and Bengal tigers (Driscoll *et al*., 2009) argues against this route. Whilst the elevation of the southern route falls within the limits of known tiger dispersal [tigers have been found at altitudes of 4000m in Bhutan (Sangay & Wangchuk, 2005)], the Hindu Kush mountain range extends directly through the modelled corridor [*Figure 4(a-c),*] and may have acted as a significant barrier to tiger dispersal. A higher treeline in northern Bhutan [4750m (Miehe *et al*., 2007)] compared with the central Hindu Kush [3200m (Schickhoff, 2005)] could account for the presence of tigers at higher altitudes in Bhutan. Alternatively, given the strength of habitat suitability through the southern corridor, colonisation of the Caspian may have occurred through simultaneous dispersal via Siberia and the northern corridor and through the southern corridor [*Figure 3(8+9)*].

The MaxEnt models indicate suitable tiger habitat through the Indian Subcontinent during the LGM, and this is supported by evidence of tigers in Sri Lanka 16.5ka [*Figure 2(a)*], a time when lower, glacial, sea levels would have allowed colonisation from India (Manamendra-Arachchi et al., 2005). Whilst the models appear to show a connection between Korea and Southern Japan during the LGM, which could have allowed the movement of tigers, a 20km wide Korean/Tsushima Strait existed between the two landmasses throughout the LGM (Park et al., 2000) which is 33% further than tigers have been known to swim across seas, even under more benign conditions, and was likely too inhospitable a barrier to allow dispersal. Therefore, it is probable that the extinct Japanese tiger colonised the islands during a previous glacial period, when eustatic sea levels were lower than at the LGM (Rohling *et al*., 1998), and thus represents a distinctly different population from mainland tigers, as proposed by Kitchener & Dugmore (2000).

The extent of the Toba super-eruption ashfall, in relation to likely suitable tiger habitat at the time, can be seen in *Figure 3(1)*. whilst it is probable that many mammal species were able to survive the Toba eruption in geographically isolated refugia (Prothero, 2004; Louys, 2007), large carnivorans, such as the tiger, are likely to have been particularly susceptible to extinction if confined to limited areas, owing to their requirement for large home range sizes (O'Regan et al., 2002), a notion supported by a demographic reconstruction of the tiger during the late Pleistocene (Wilting *et al*., 2015). It is conceivable that direct damage caused

by the YTT, and rapid cooling and drought in the following decades, were responsible for the eradication of tigers from most, if not all of, Sumatra and the Malay Peninsula, and might have significantly impacted populations farther afield.

In addition to the direct and indirect environmental effects of the Toba eruption, tigers could have been affected by cooler, stadial conditions lasting around 1000 years after the eruption (Rampino & Self, 1992; Harris, 2008). A subsequent shift from interglacial to glacial conditions, (or from present-day to LGM tiger distributions by proxy) and its impact on woodlands would have limited the recovery of tigers in the areas affected by Toba [*Figure 3 - ~73ka(+1k)*]. An exception could have occurred within the Sunda Islands, where a glacially-driven decrease in global sea level during this time (Chappell & Shackleton, 1986) may have allowed the recolonisation of Sumatra from Java through temporary land bridges. Even if this opportunity was missed, tigers swim well and have been known to cross rivers 29 km wide and expanses of sea 15km wide (Kitchener, 1999), so it is conceivable that tigers could have swum between the Sunda Islands even after land bridges were inundated. Indeed, it is likely that tigers swam between Java and Bali into recent times (Kitchener, 1999) a notion supported by the genetic similarity between Javan and Bali tigers, and high morphological affinity between all Sunda Island tigers (Wilting et al., 2015) [*Figure 1(d)*].

Southern China/Southeast Asia and the eastern Sunda Islands of Java and Bali represent the 'core' areas of the modelled tiger range that have endured through both glacial and interglacial periods, and they lie outside the major ash fallout zones of the Toba eruption. However, a Sunda Island refugium is unlikely because of the phylogenetic position of the putative South China tiger (*P.t. amoyensis*), which suggests it is ancestral to other tiger lineages (Luo *et al*., 2004; Driscoll *et al*., 2009; Wilting *et al*., 2015), and indicates a population collapse and re-dispersal out of southern China/southeastern Asia during the Late Pleistocene, including into the Sunda Islands.

At the LGM, and into postglacial times until the 19th century,, suitable habitats existed in India for both the ancestors of modern lions and tigers [*Figure 3(6)*], enabling them to be sympatric, but in separate habitats. Indeed, the latest phylogeographical study of lions suggest that they entered the Indian subcontinent around the LGM (Barnett *et al*., 2014), because of more arid conditions at that time. The disappearance of Eurasian cave lions from northern Asia around 11ka (Barnett *et al*., 2009) coincided with climatic and vegetation shifts that

favoured the western dispersal of tigers throughout this region, so it is difficult to assess the extent to which competition may have limited one species or the other. The presence of both modern lions and Eurasian cave lions in the Near East may have reinforced the separation between tiger populations of the Indian Subcontinent and the Caspian region.

The models presented here indicate a recent northern dispersal of tigers, beginning after the LGM, and before the Mid-Holocene Climatic Optimum. It is likely that the early rise of civilisations in China, especially along the rivers of the Huanghe catchment over 8ka (Kong, 1992) resulted in local extirpations of tigers and thus reduced contact, and division between northern continental tigers (Caspian and Amur populations) and southern continental tigers (remaining mainland populations). Major early impacts on Chinese tiger populations are likely because of the danger posed to both humans and livestock, their prized fur and use in traditional medicine. This is supported by studies which show that Amur/Caspian populations are genetically close to Indochinese tigers (Driscoll *et al*., 2009; Wilting *et al*., 2015) [*Figure 1(d)*], suggesting that there has been insufficient time for any local genetic differentiation despite current isolation of northern populations. However, given their dispersal outside of the core Late Pleistocene habitat of southern Asia, their adaptation to a temperate ecosystem, and their longer term separation compared to more recently fragmented populations of mainland tiger, the results complement the recognition of separate conservation management of northern continental tigers from that of southern continental tigers, as proposed by Wilting *et al*., (2015).

The modelling presented indicates there has been significant separation between continental and Sunda Island populations of tigers since the Last Glacial Maximum, which led to significant population differentiation.. This has been proposed by previous genetic, morphological and biogeographical studies (Cracraft et al., 1998; Kitchener, 1999; Kitchener & Dugmore, 2000; Kitchener & Yamaguchi, 2010; Mazák, 2010). There is a clear disparity between current classifications that recognise up to nine tiger subspecies and the contiguity between continental tiger populations during the period of the adaptive radiation of modern populations over the last ca.100ka. In comparison to the contiguity of continental tigers, and their proposed post LGM northward radiation, it is suggested that Asiatic lions constitute the same Evolutionary Significant Unit as North African lions, despite radiating to their currently fragmented position ~21ka (Barnett *et al*., 2014). While biogeographical models are not taxonomic tools for deciding the validity of species and subspecies, they provide a deeper

time framework against which to judge the significance of genetic and morphological differences between the fragmented populations of today. Thus, they can inform current conservation strategies for endangered widespread species, such as the tiger, and be used to question traditional taxonomies that are based on poor levels of evidence. The scale-dependent nature of taxonomy (Crandall et al., 2000) benefits from an understanding of the changing relationships between populations through space and time. This broader biogeographical approach adds important perspectives to current debates about what we are trying to conserve both in the wild and captivity, and adds additional scientific weight to arguments for more pragmatic and realistic approaches to conservation.

Conclusions

Using a niche modelling approach, the probable dynamic range shifts of the tiger during the Late Pleistocene and Holocene are reconstructed, a period when genetic data suggest that modern populations colonised southern and eastern Asia. Throughout this period the tiger's core distribution was in southeastern Asia, southern China and eastern Sundaland, and that tigers colonised northeastern and central Asia from southeastern Asia. Owing to continuing geographical contiguity during glacial-interglacial cycles, which allowed for continuing potential gene flow, corroborated by recent molecular studies (Wilting *et al*., 2015), there is a clear disparity with classifications that recognise six subspecies among mainland populations. The separation of the Sunda Islands from the mainland through sea-level rise since the LGM, is consistent with the recognition of potential island population differentiation.

The notion that mainland tiger populations remained contiguous with each other through the Late Pleistocene until Holocene anthropogenic impacts fragmented populations, has significant implications for management and conservation. The recognition of only two tiger subspecies (Sunda - *Panthera tigris sondaica* and Continental tigers - *Panthera tigris tigris*), with the later split into northern and southern populations, which are proposed as separate continental management units (Wilting *et al*., 2015), would benefit current tiger conservation efforts by allowing more genetic interchange between currently isolated and limited populations. The biogeographical modelling presented here supports only a significant differentiation between Continental and Sundaland tiger populations and thereby offers

important insights relevant to both future research and discussions on current tiger conservation.

Supplementary Information

Author Contributions

This chapter has been published in *Diversity and Distributions* as a collaborative paper (Cooper et al., 2016). The data was collated, analysis performed and chapter written by myself. Andrew Kitchener and Andrew Dugmore and Bruce Gittings helped design the research. Andreas Wilting provided modelling advice. Anne Scharf contributed data.

Appendix S1

Ecological niche model evaluation, parameter selection and projection process to past conditions.

Appendix S1 – Model parameterisation and evaluation

Here the model setup is outlined, and the effects of changing MaxEnt model parameters are evaluated. Model parameterisation is based upon literature best practices, evaluation of recognised metrics, and the likely effects of the environmental variables on the distribution of the tiger. Projections to palaeo-climates and final map outputs are also visualised.

Contents

Model inputs

The background extent used for modelling is reduced to potential tiger range, and thereby excludes islands not connected to the continent during the Last Glacial Maximum (LGM), Africa, east of Beringia, and Western Europe. 511 tiger localities from four sources (detailed in the body of the chapter) are available for model creation in conjunction with WorldClim bioclimatic variables (Hijmans et al., 2005). Highly correlated variables are removed from the analysis (correlation $\geq$ 0.8, calculated using ArcGIS Band Collection Statistics (ArcMap v10.1)) to reduce over parameterisation (Buermann et al., 2008; Garcia-Porta et al., 2012)

Model Parameterisation

MaxEnt is chosen for species distribution modelling based on its performance over other techniques in presence only scenarios (Elith et al., 2006; Hernandez et al., 2006; Phillips et al., 2006). MaxEnt is used in its capacity as a poisson point process model (Renner et al., 2015) by unchecking "remove duplicate presence records" and "add samples to background" and by viewing the raw output values. An assumption of the poisson point process model is that point locations are independent of one another – which is likely given the number of independent sources the locality points have been gathered from, and the wide spatial coverage of the locality points collected, which includes areas of recent historical presence such as China, the Caspian region, Java and Bali.

The effects of changing the number of background points, regularisation multiplier and correlated variables on model performance are tested. In comparisons, unless otherwise stated, the following parameters are chosen:

- Regularisation multiplier set to 2, in accordance with (Radosavljevic & Anderson, 2014) who found values higher than default (1) yielded substantially lower over-fitting.
- Background points set to 100,000, as the effective number quadrature points for sufficient convergence of the log-likelihood is likely significantly higher than the default (10,000), and has been found to be closer to 100,000 in previous studies (Renner et al., 2015).
- Features set to "Autofeatures" to allow fitting of Linear, Quadratic, Hinge, Threshold and Product models.

- All localities are used
- 9 bioclimatic variables (correlations <0.8) used as covariates:
    - BIO1: annual mean temperature
    - BIO2: mean diurnal range
    - BIO3: isothermality
    - BIO8: mean temperature of the wettest quarter
    - BIO15: precipitation seasonality
    - BIO16: precipitation of the wettest quarter
    - BIO17: precipitation of the driest quarter
    - BIO18: precipitation of the warmest quarter
    - BIO19: precipitation of the coldest quarter

For comparative plots (*Figure 1, 3+4*), projected models are displayed using a Maximum Training Sensitivity plus Specificity (MTSS) threshold based on the MaxEnt logistic output, so as to better visualise differences in habitat suitability (although note that final projections are presented in raw output). Models are projected onto present day data (as used for model creation) as well as the Last Glacial Maximum CCSM4 data, so show the effect of changing model parameters on novel environmental conditions. Mean AUC values are provided from 10-fold cross-validation.

Regularisation Multiplier (RM)

Mean AUC values:

RM = 1: **0.847**

RM = 2: **0.843**

RM = 4: **0.840**

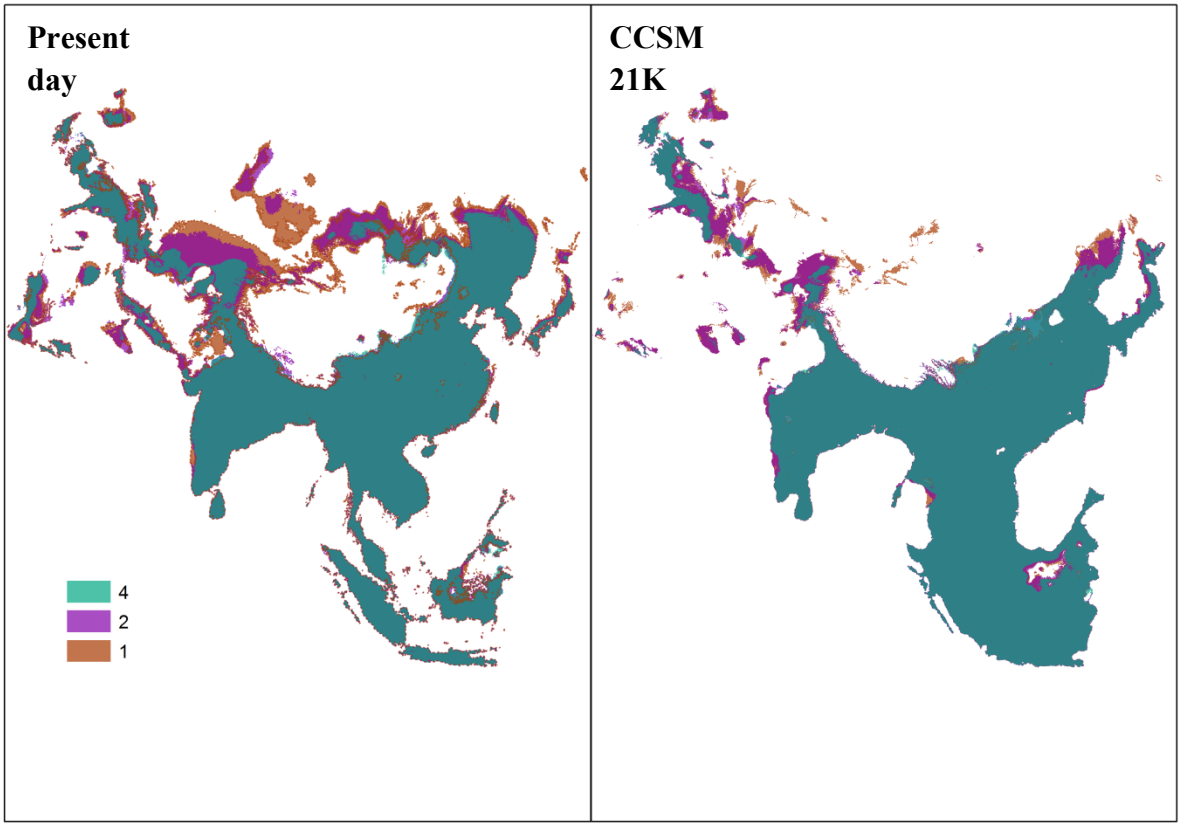


Figure 5: RM effects on projected models for the present day, and LGM under the CCSM4 bioclimatic scenario. Adjusting the RM has little effect upon modelled tiger habitat suitability for the present day or LGM over the majority of the study area. The largest differences in projected models occur in the Caspian region for present day conditions, where lower RM values indicate a larger area of suitable habitat**.**

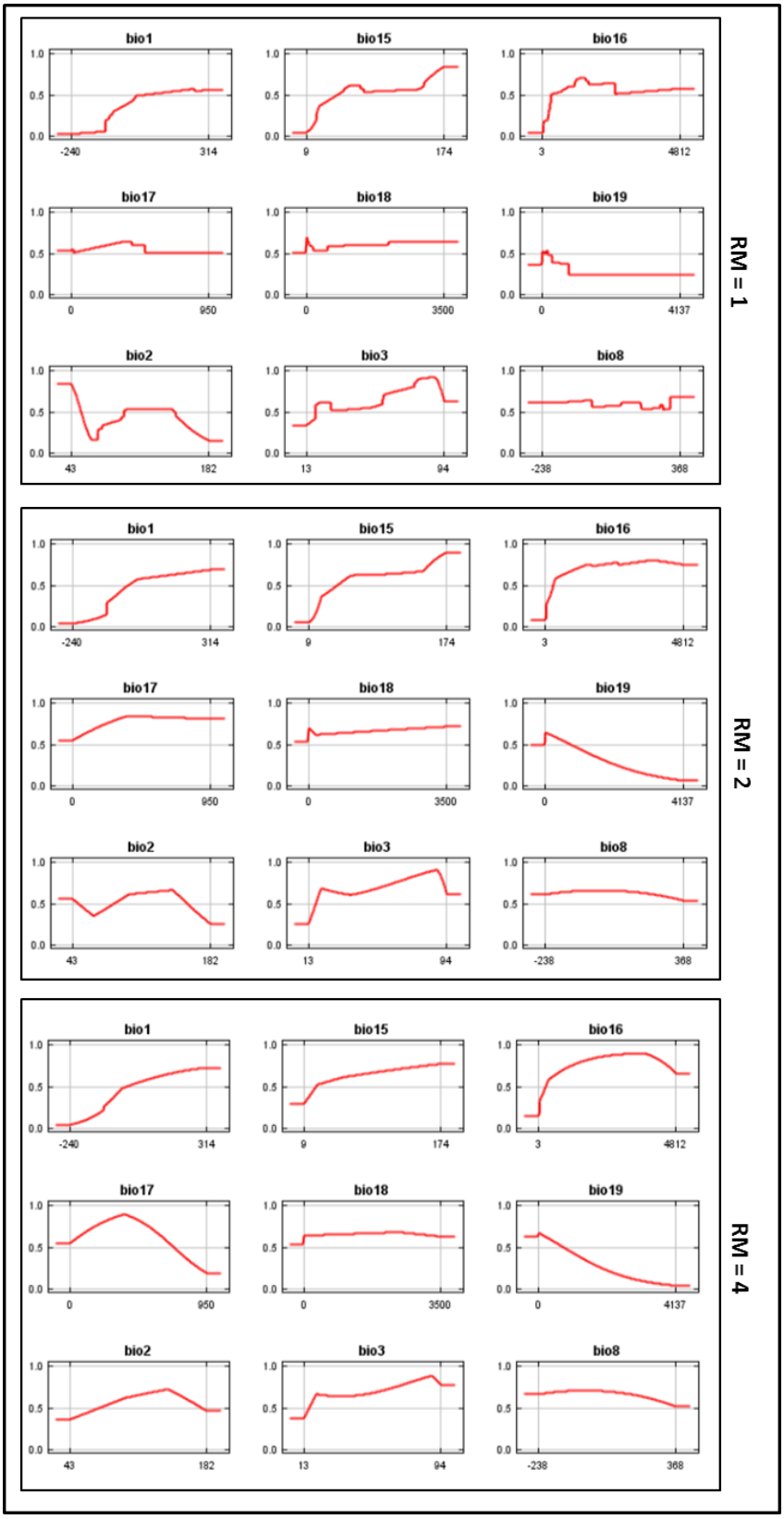


Figure 2: Response curves based on varying the RM. Higher RM values yield smoother response curves. RM = 1 appears to overfit to the data, whilst RM = 4 exhibits simpler response curves. Final models are created with RM = 2

Background Points (BP)

Mean AUC values:

#BP = 10,000: **0.844**

#BP = 40,000: **0.844**

#BP = 100,000: **0.843**

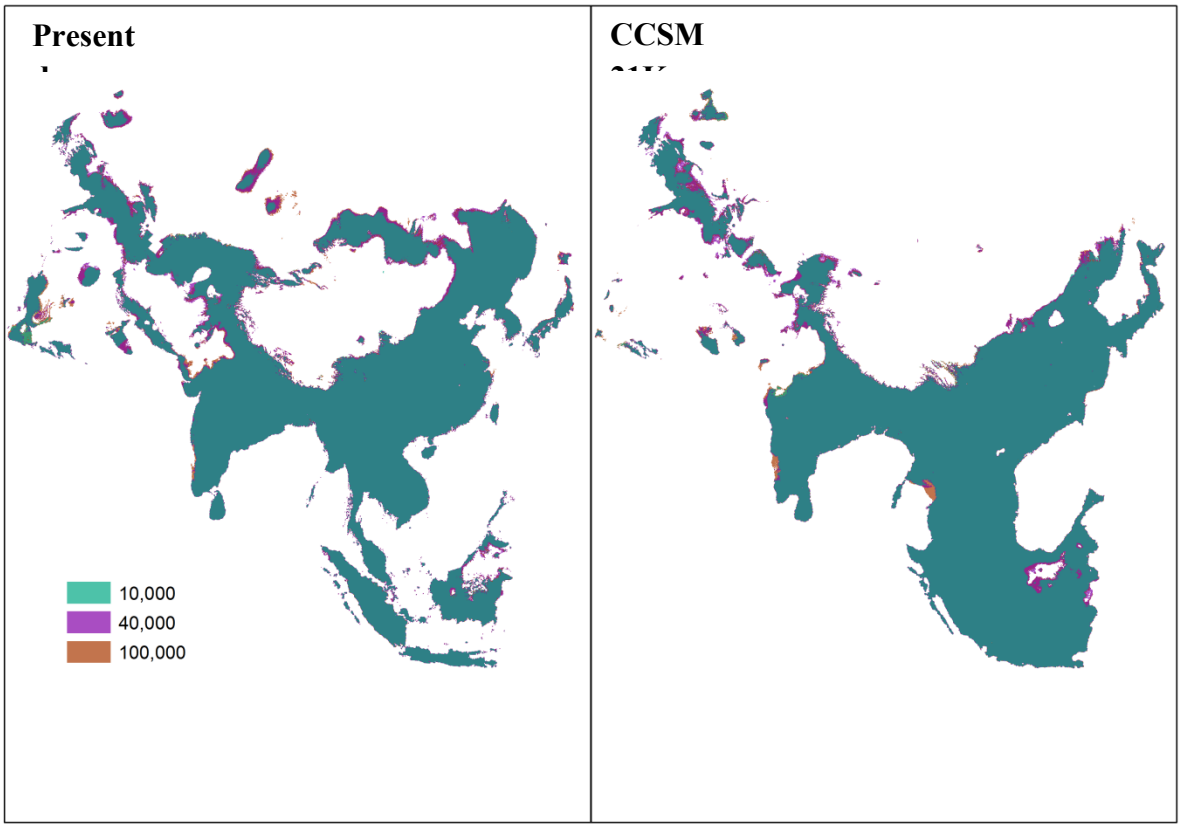


Figure 3: Effects of adjusting the number of BP on projected models for the present day, and LGM under the CCSM bioclimatic scenario. The number of BP has had a negligible effect on modelled habitat suitability. For the final model 100,000 BP are chosen, given that a higher number BP will result in a more accurate estimate of the model likelihood (Renner *et al*., 2015).

Auto-correlated localities

Mean AUC values:

All localities: **0.843**

Spatially filtered localities: **0.844**

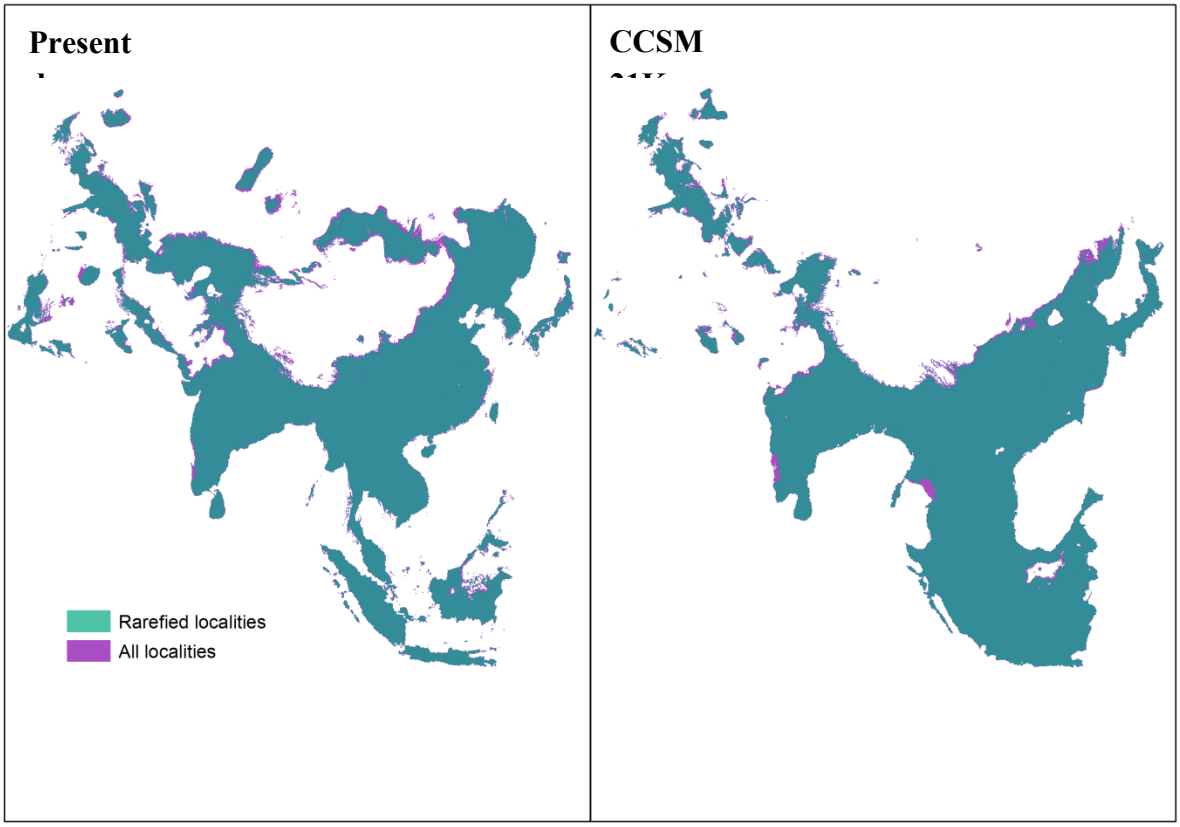


Figure 4: Difference between using all localities and using spatially rarefied localities on the projected model. Using the SDMtoolbox for ArcGIS (Brown, 2014), tiger locality records were rarefied based on variable pairwise distances dependent on climate variable heterogeneity. A measure of climate heterogeneity was created using the selected WorldClim bioclimatic variables. Occurrence data were rarefied based on five classes of bioclimatic heterogeneity, separated through natural breaks, which separate values where large changes occur (de Smith et al., 2006). In climatically homogenous landscapes, the maximum distance for the removal of spatially similar localities was set at 100km, while in climatically heterogeneous landscapes the minimum distance was set at 5km. Differences between using all localities and spatially rarefied localities is minimal. Final models are created using all localities, to be run as a point process model.

Final Model

Model settings:

510 presence records used for training (all localities)

100,000 points used to determine the MaxEnt distribution (background points).

Environmental layers used (all continuous): bio1 bio2 bio3 bio8 bio15 bio16 bio17 bio18 bio19

Regularization values: linear/quadratic/product: 0.050, categorical: 0.250, threshold: 1.000, hinge: 0.500

Feature types used: hinge product linear threshold quadratic

responsecurves: true

outputformat: raw

removeduplicates: false

betamultiplier (Regularisation multiplier): 2.0

maximumbackground: 100000

addsamplestobackground: false

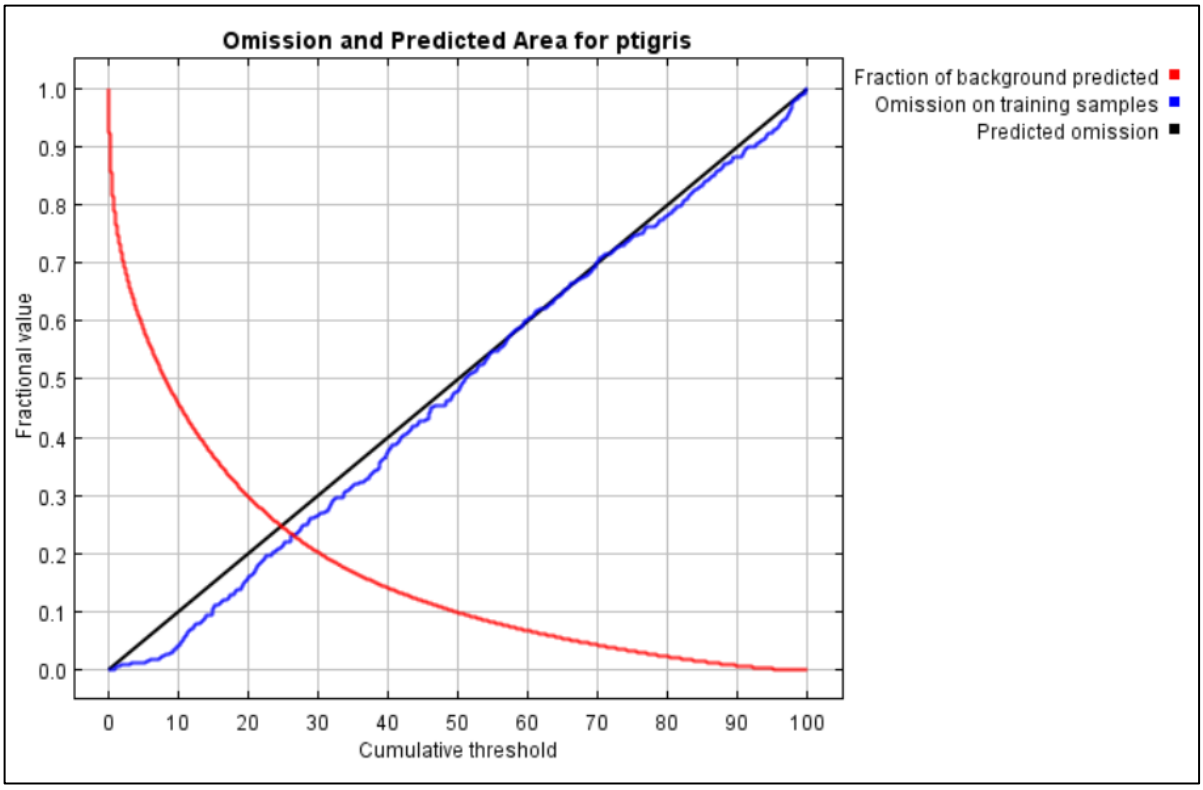


Figure 5: Final MaxEnt model output of the omission rate and predicted area as a function of the cumulative threshold. Omission rate calculated on the training presence records (all 511 records used for final model).

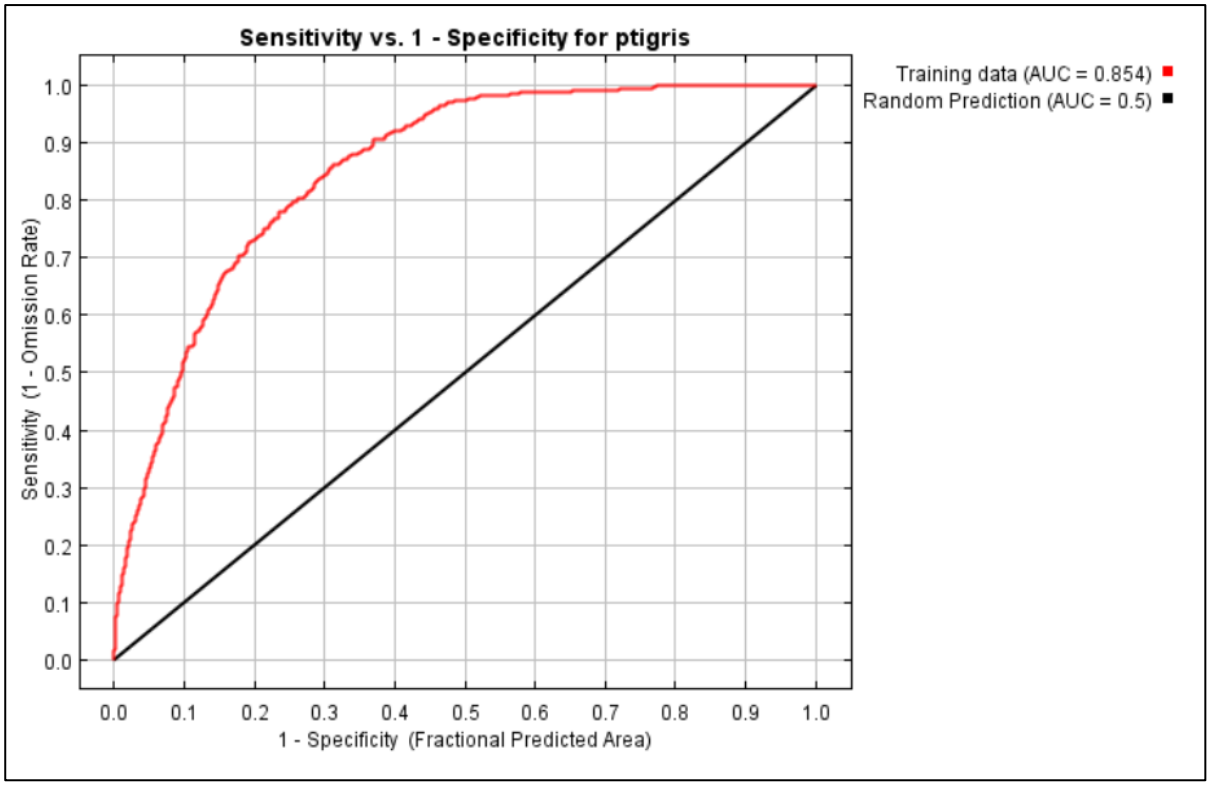


Figure 6: Receiver operating characteristic (ROC) curve.

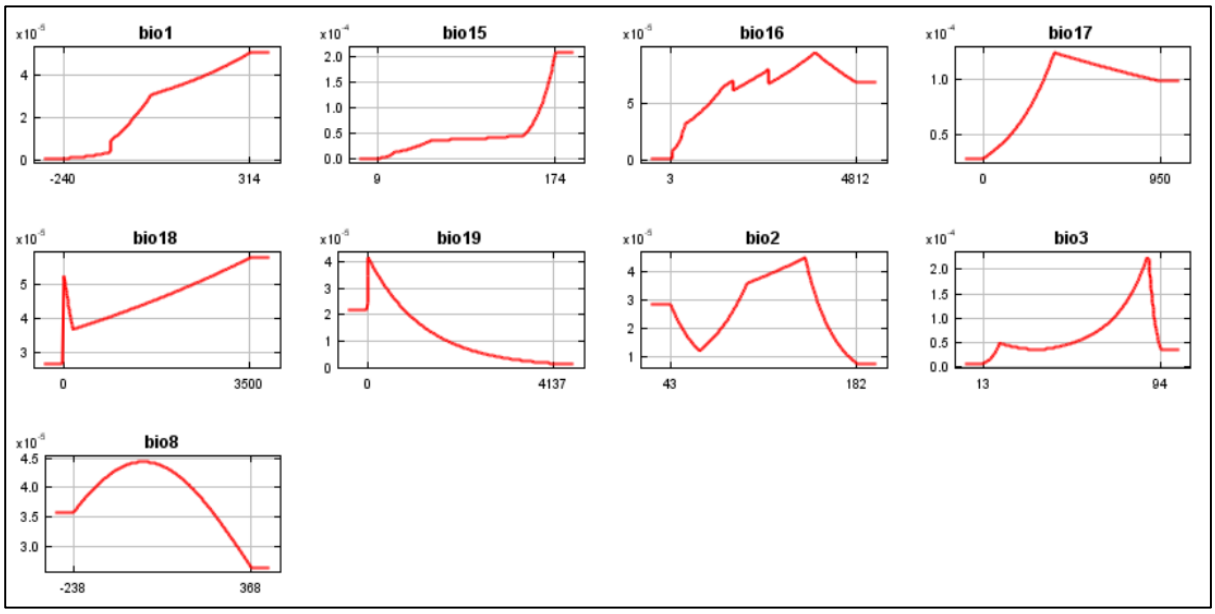


Figure 7: Response curves showing the effect of individual environmental variable upon the MaxEnt prediction, keeping all other variables at their average sample value.

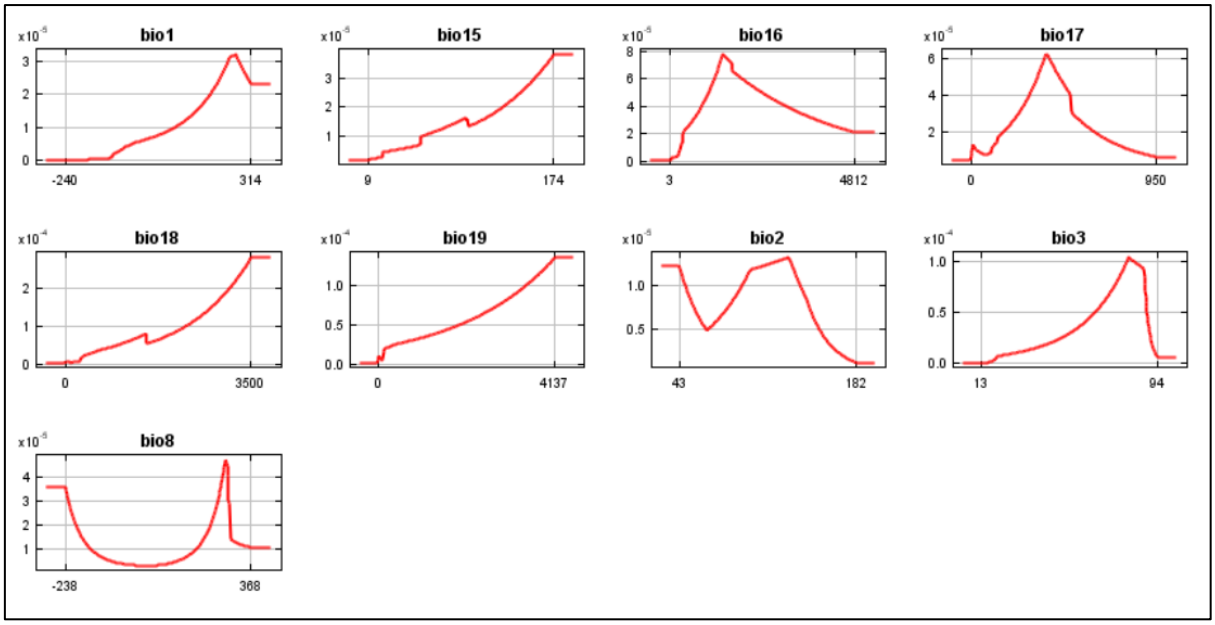


Figure 8: Response curves of MaxEnt models fitted based on the corresponding variable only.

Table 1: Estimates of the relative contributions of the bioclimatic variables in the final MaxEnt model.

Variable	Percent contribution	Permutation importance
bio16	67.4	28.3
bio1	12.6	28.6
bio3	9.2	11.9
bio15	3.9	19
bio2	3.5	5.1
bio19	1.2	2.7
bio18	1.1	2.2
bio8	0.7	0.3
bio17	0.5	1.8

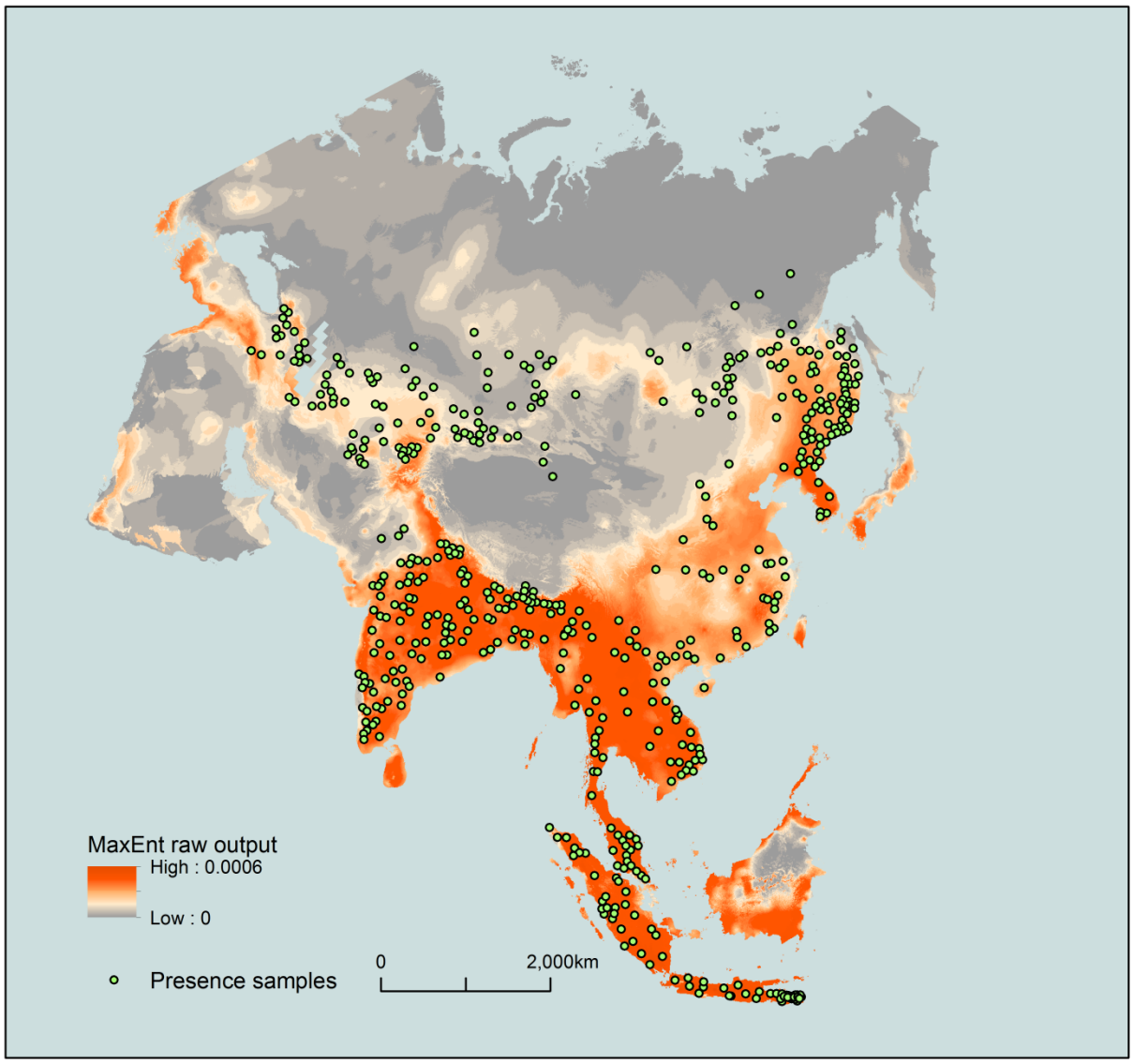


Figure 9: Final model projected onto present day conditions, with training presence samples overlaid.

Mid Holocene / LGM Ensemble Forecasting

Considerable variation between coupled general circulation model-derived distribution models has been noted in previous studies, with differences attributed to the different assumptions of each model (Garcia-Porta *et al*., 2012; Rebelo *et al*., 2012; Gassert *et al*., 2013), however the models [*Figure 10(a-d)+Figure 11(a-d)*] show that the latest iterations of models based on CMIP5 data provide considerably greater consistency. MESS grids [*Figure 10(a-d)-inserts+Figure 11(a-d)-inserts*] show negligible effects on projected outputs. Unfavourable vegetation (desert and tundra) from BIOME 4 (Kaplan *et al*., 2003) is presented upon projected outputs as a measure of validation. A mean model for final

projections is created [*Figures 10(e)+11(e)*] as the habitat suitability from each general circulation model is deemed as equally likely.

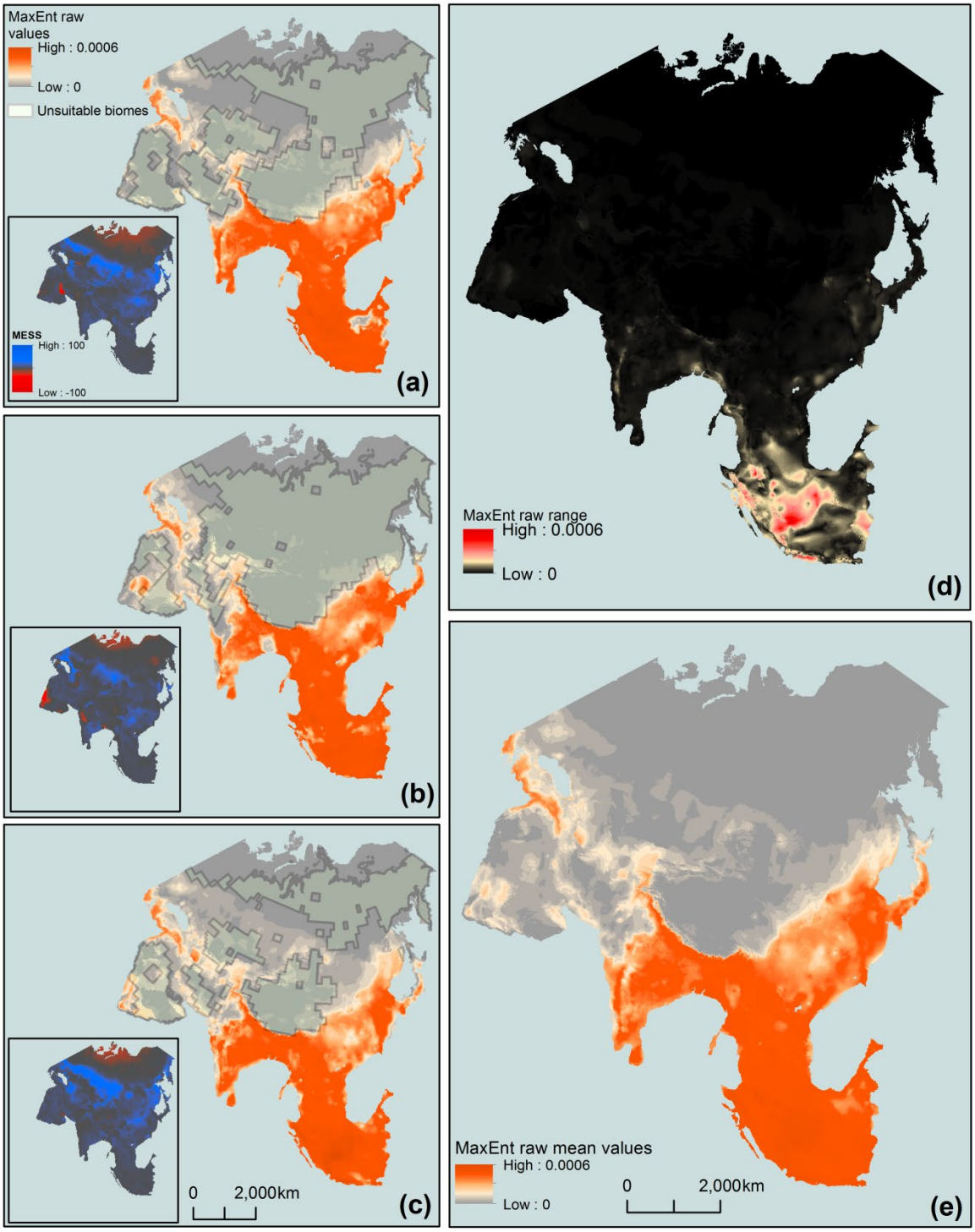


Figure 6: Final model projected onto Last Glacial Maximum bioclimatic conditions from CCSM (a), MIROC-ESM (b) and MPI-ESM-P (c). BIOME4 unsuitable vegetation (desert+tundra) is presented over models – strong habitat suitability has not occurred within unfavourable vegetation. MESS grids show no large effect of environmental variables outside the range of the training data upon the model projections (insets (a)-(c)). The range of raw values between all three models (d) is high

within areas where all projections modelled high suitability. The final LGM projection (e) is the mean value of each general coupled circulation model projection.

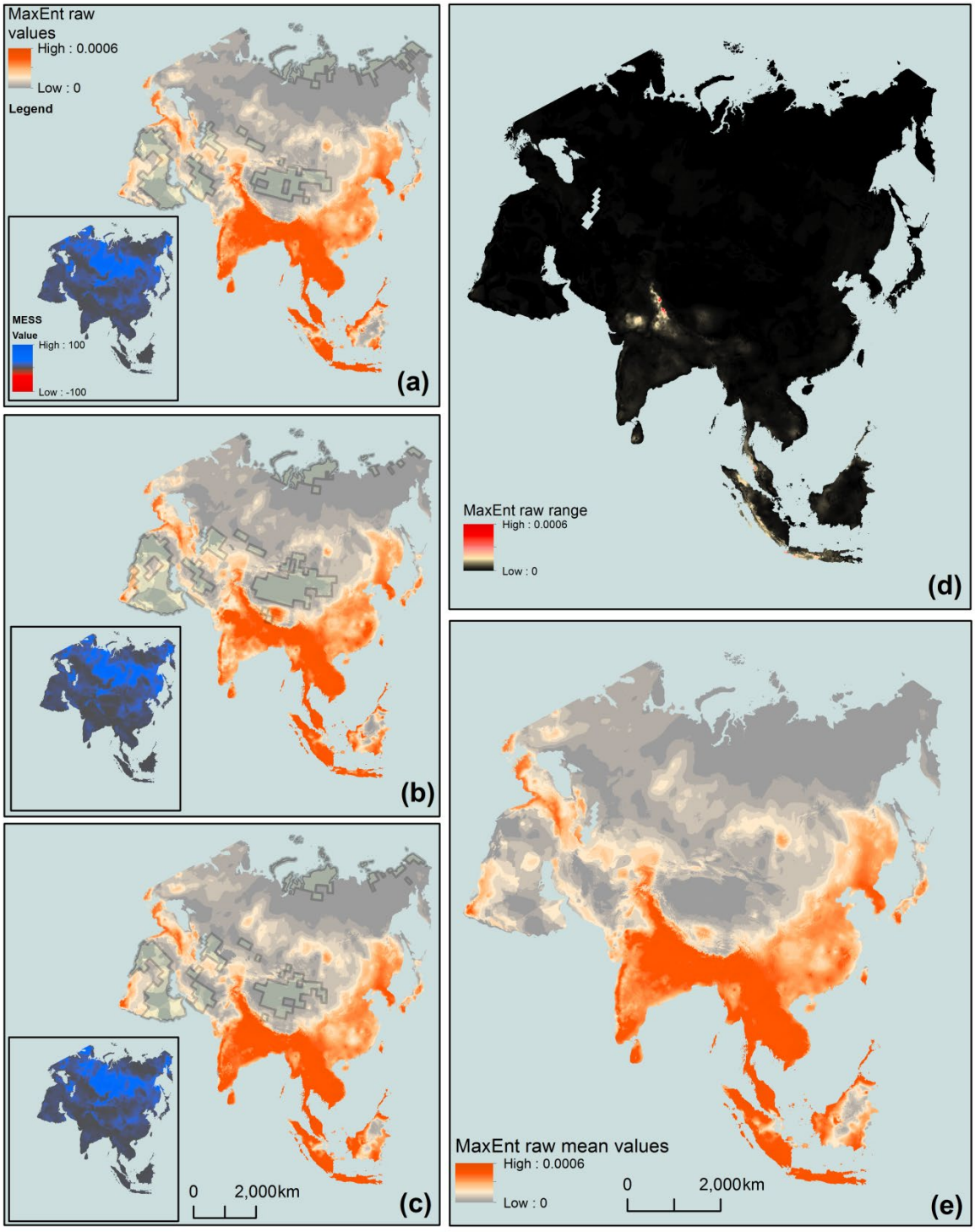


Figure 7: Final model projected onto Mid-Holocene bioclimatic conditions from CCSM (a), MIROC-ESM (b) and MPI-ESM-P (c). BIOME4 unsuitable vegetation (desert+tundra) is presented over models – strong habitat suitability has not occurred within unfavourable vegetation. MESS grids show no large effect of environmental variables outside the range of the training data upon the model projections (insets (a)-(c)). The range of raw values between all three models (d) is high within areas where all projections modelled high suitability. The final Mid-Holocene projection (e) is the mean value of each general coupled circulation model projection.

Final model (MaxEnt) compared with Boosted Regression Trees

The focus in this study has been the correct parameterisation of a MaxEnt model under a poisson point process framework, as a contemporary method of dealing with presence-only data (Renner *et al*., 2015). Here MaxEnt is compared with Boosted Regression Trees (BRT) – a machine learning technique traditionally applied to presence-absence data (Elith et al., 2008). Whilst BRT may be applied to presence only data in equivalence to an inhomogeneous poisson process model (Fithian & Hastie, 2013), BRT are applied under naïve logistic regression through its default settings in the biomod2 package in R (R Core Team, 2015) – this enables comparison between a best practice model (final MaxEnt model) and a readily available and easily implemented alternative. For comparison, due to biomod2 settings, MaxEnt and BRT models are compared via logistic output. Projected comparisons are shown in *Figures 12 +13* – the same conclusions regarding tiger distributions are made from both models.

<u>BRT settings:</u>
Environmental variables and tiger localities are the same as MaxEnt final model
Naïve-absence points = 100,000
Distribution = Bernoulli,
Number of trees = 2500,
Interaction depth (maximum depth of variable interactions) = 7,
Minimum number of observations in the trees terminal nodes = 5,
Shrinkage = 0.001,
Bag fraction = 0.5,
Train fraction = 1,

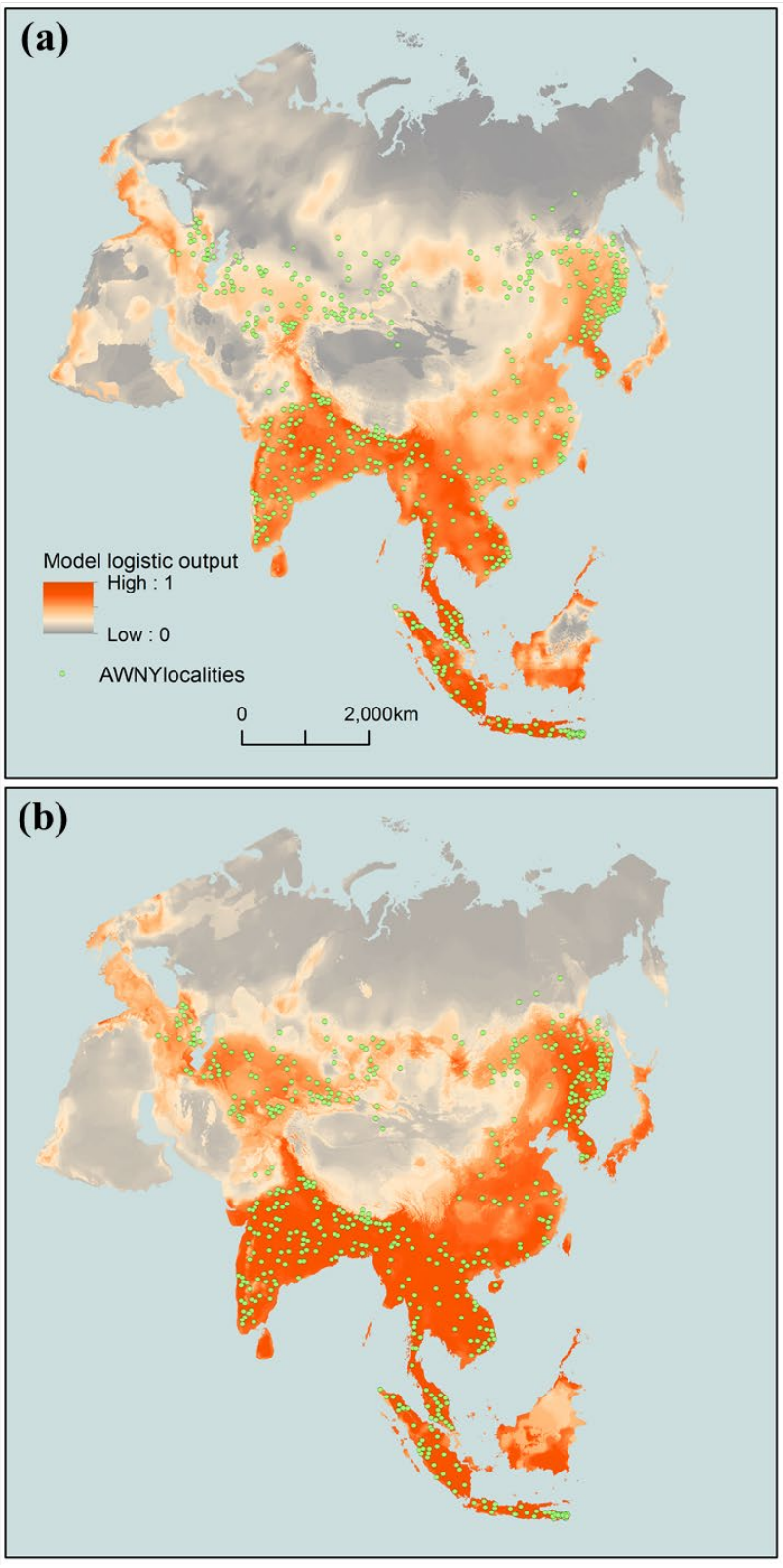


Figure 8: MaxEnt (a) and BRT (b) logistic output for present day bioclimatic conditions. BRT shows greater habitat suitability across the range of the tiger in comparison with the MaxEnt model. Whilst BRT modelled suitability is higher, it is largely consistent with the MaxEnt model in terms of the geographic locations of modelled suitable habitat. Overall conclusions of potential present tiger range do not differ between models.

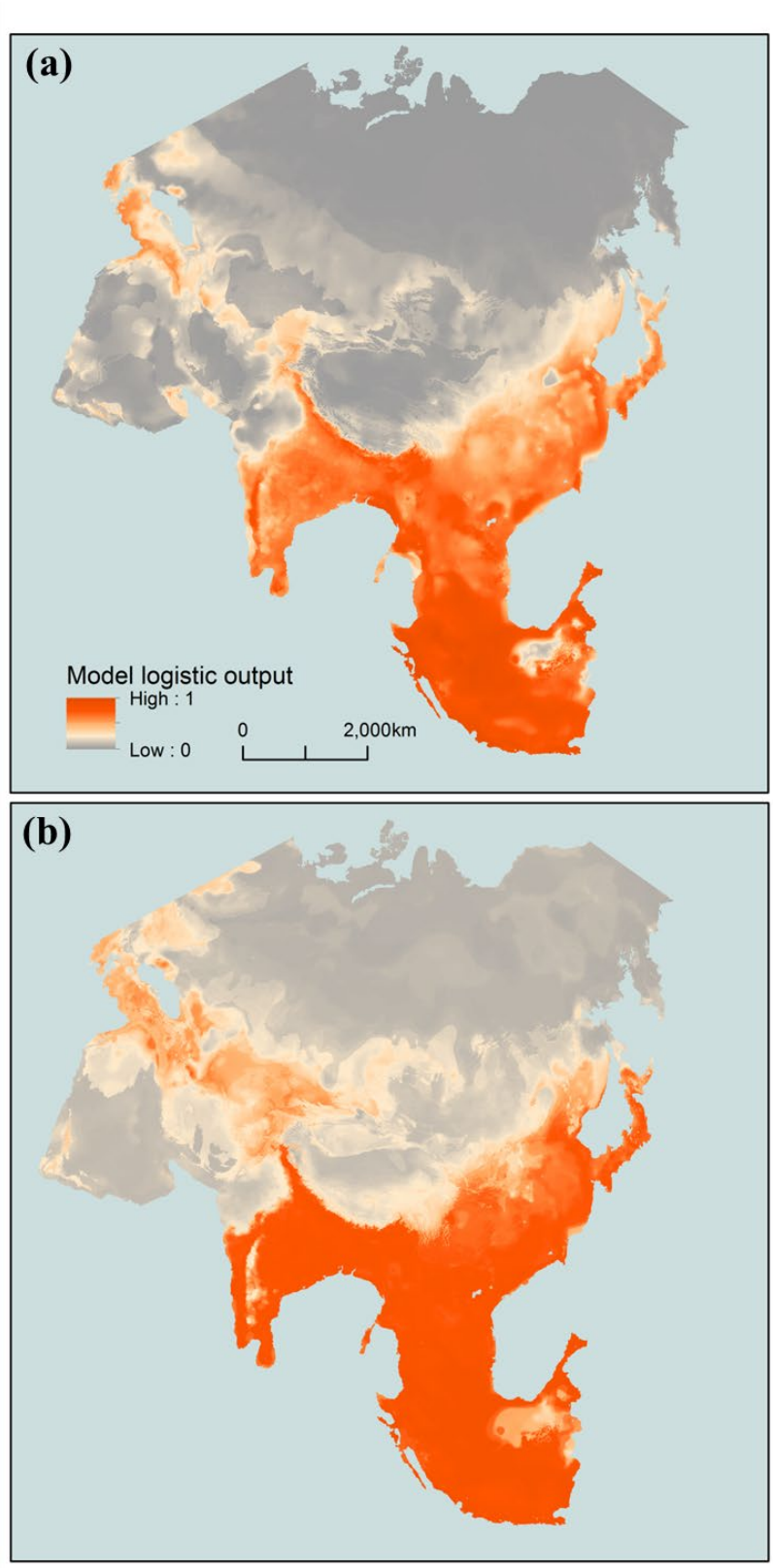


Figure 9: MaxEnt (a) and BRT (b) logistic output for CCSM4 LGM bioclimatic conditions. As with present models (*Figure 12*), BRT shows greater suitability across the range of the tiger than the MaxEnt model. By comparing between (a) and (b) it may appear as though the BRT model supports a potential LGM population within the Caspian. However, the vector of change between present conditions and the LGM for both MaxEnt and BRT models is the same - decreased habitat suitability modelled in the northern ranges, including the Caspian region; and therefore overall conclusions over likely tiger range remain unchanged.

Low Predicted Habitat Suitability on Present Day Borneo

Borneo differs from neighbouring Sumatra in predicted suitability for the tiger under present environmental conditions. To assess the underlying cause of the differences in habitat suitability, the explain tool in MaxEnt was implemented (*Figure 14*). The explain tool is implemented with linear, quadratic and hinge features only (explain tool does not allow use of threshold or product features) – all other model settings match those of the final model. In comparing low predicted suitability in Borneo[*Figure 14(a)*], marginal habitat suitability in Borneo [*Figure 14(b)*] and high habitat suitability in neighbouring Sumatra [*Figure 14(c)*] the variable which crosses a significant environmental suitability gradient is bio15 [Precipitation Seasonality (Coefficient of Variation)]. In Borneo, where precipitation seasonality is low, predicted habitat suitability is low. The lack of seasonality, caused by heavy rains which disrupt draught [see differences in bio17: Precipitation of the Wettest Quarter *(Figure 14)*] is linked to the absence of large carnivores in Borneo through its effect on flowering and fruiting of dipterocarp forests (Meijaard, 2004; Wong et al., 2005).

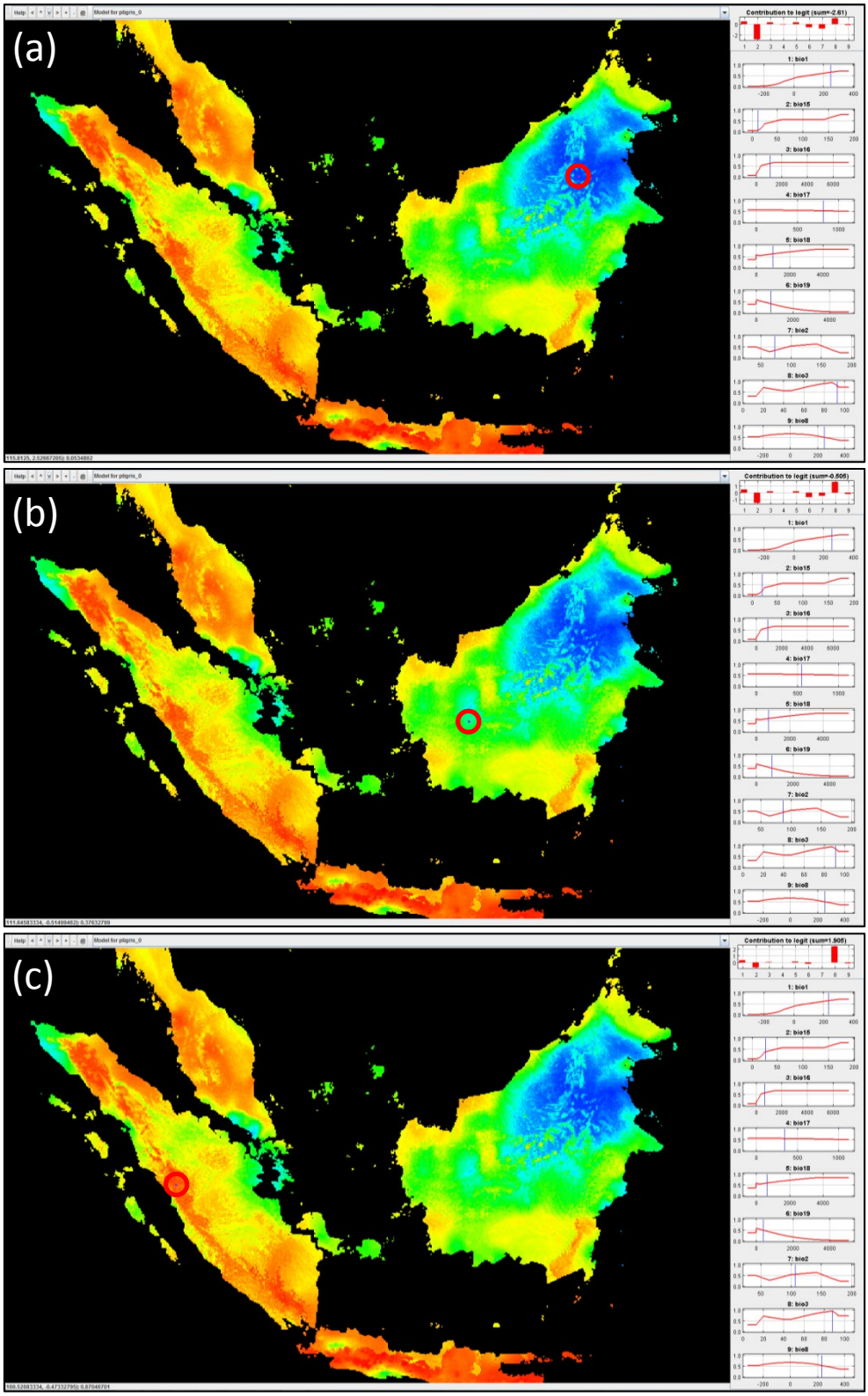


Figure 14 – MaxEnt explain tool focussed on Sumatra and Borneo. Warm map colours represent high, and cold colours represent low modelled habitat suitability for the tiger under present conditions. The effect of environmental variables is explored at point locations in northern Borneo (a), western Borneo (b) and Sumatra (c) for low (a), marginal (b) and high (c) modelled habitat suitability.

References

Acharyya S.K. & Basu P.K. (1993) Toba ash on the Indian Subcontinent and its implications for correlation of Late Pleistocene Alluvium. *Quaternary Research*, **40**, 10–19.

Barnett R., Shapiro B., Barnes I., Ho S.Y.W., Burger J., Yamaguchi N., Higham T.F.G., Wheeler H.T., Rosendahl W., Sher A. V, Sotnikova M., Kuznetsova T., Baryshnikov G.F., Martin L.D., Harington C.R., Burns J.A., & Cooper A. (2009) Phylogeography of lions (Panthera leo ssp.) reveals three distinct taxa and a late Pleistocene reduction in genetic diversity. *Molecular ecology*, **18**, 1668–77.

Barnett R., Yamaguchi N., Shapiro B., Ho S.Y.W., Barnes I., Sabin R., Werdelin L., Cuisin J., & Larson G. (2014) Revealing the maternal demographic history of Panthera leo using ancient DNA and a spatially explicit genealogical analysis. *BMC Evolutionary Biology*, **14**, 70.

Bay R.A., Ramakrishnan U., & Hadly E.A. (2014) A call for tiger management using "reserves" of genetic diversity. *Journal of Heredity*, **105**, 295–302.

Braconnot P., Otto-Bliesner B., Harrison S., Joussaume S., Peterchmitt J.-Y., Abe-Ouchi a., Crucifix M., Driesschaert E., Fichefet T., Hewitt C.D., Kageyama M., Kitoh a., Laîné a., Loutre M.-F., Marti O., Merkel U., Ramstein G., Valdes P., Weber S.L., Yu Y., & Zhao Y. (2007) Results of PMIP2 coupled simulations of the Mid-Holocene and Last Glacial Maximum – Part 1: experiments and large-scale features. *Climate of the Past*, **3**, 261–277.

Brown J.L. (2014) SDMtoolbox: a python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. *Methods in Ecology and Evolution*, **5**, 694–700.

Buermann W., Saatchi S., Smith T.B., Zutta B.R., Chaves J.A., Milá B., & Graham C.H. (2008) Predicting species distributions across the Amazonian and Andean regions using remote sensing data. *Journal of Biogeography*, **35**, 1160–1176.

Chappell J. & Shackleton N.J. (1986) Oxygen isotopes and sea level. *Nature*, **324**, 137–140.

Cooper D.M., Dugmore A.J., Gittings B.M., Scharf A.K., Wilting A., & Kitchener A.C. (2016) Predicted Pleistocene-Holocene range shifts of the tiger (Panthera tigris). *Diversity and Distributions*, **22**, 1199–1211.

Costa A., Smith V.C., Macedonio G., & Matthews N.E. (2014) The magnitude and impact of the Youngest Toba Tuff super-eruption. *Frontiers in Earth Science*, **2**, 1–8.

Cracraft J., Feinstein J., Vaughn J., & Helm-Bychowski K. (1998) Sorting out tigers (Panthera tigris): mitochondrial sequences, nuclear inserts, systematics, and conservation genetics. *Animal Conservation*, **1**, 139–150.

Crandall K.A., Bininda-Emonds O.R.P., Mace G.M., & Wayne R.K. (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution*, **15**, 290–295.

Dinerstein E., Loucks C., Heydlauff A., Wikramanayake E., Bryja G., Forrest J., Ginsberg J., Klenzendorf S., Leimgruber P., O'Brien T., Sanderson E., Seidensticker J., & Songer M. (2006) *Setting Priorities for the Conservation and Recovery of Wild Tigers: 2005-2015. A User's Guide.* WWF, WCS, Smithsonian, NFWF-STF, Washington D.C., New York.

Driscoll C.A., Yamaguchi N., Bar-Gal G.K., Roca A.L., Luo S., Macdonald D.W., & O'Brien S.J. (2009) Mitochondrial phylogeography illuminates the origin of the extinct caspian tiger and its relationship to the amur tiger. *PloS one*, **4**, e4125.

Elith J., Graham C.H., Anderson R.P., Dudík M., Ferrier S., Guisan A., Hijmans R.J., Huettmann F., Leathwick J.R., Lehmann A., Lucia J.L., Lohmann G., Loiselle B.A.,

Manion G., Moritz C., Nakamura M., Nakazawa Y., Overton J.M., Peterson A.T., Phillips S.J., Richardson K., Scachetti-Pereira R., Schapire R.E., Soberón J., Williams S., Wisz M.S., & Zimmermann N.. (2006) Novel methods improve prediction of species ' distributions from occurrence data. *Ecography*, **29**, 129–151.

Elith J., Leathwick J.R., & Hastie T. (2008) A working guide to boosted regression trees. *Journal of Animal Ecology*, **77**, 802–813.

Fithian W. & Hastie T. (2013) Finite-sample equivalence in statistical models for presence-absence only data. *Annals of Applied Statistics*, **7**, 1917–1939.

Garcia-Porta J., Litvinchuk S.N., Crochet P.A., Romano A., Geniez P.H., Lo-Valvo M., Lymberakis P., & Carranza S. (2012) Molecular phylogenetics and historical biogeography of the west-palearctic common toads (Bufo bufo species complex). *Molecular phylogenetics and evolution*, **63**, 113–30.

Gassert F., Schulte U., Husemann M., Ulrich W., Rödder D., Hochkirch A., Engel E., Meyer J., & Habel J.C. (2013) From southern refugia to the northern range margin: genetic population structure of the common wall lizard, Podarcis muralis. *Journal of Biogeography*, **40**, 1475–1489.

Geffen E., Anderson M.J., & Wayne R.K. (2004) Climate and habitat barriers to dispersal in the highly mobile grey wolf. *Molecular ecology*, **13**, 2481–90.

Goodrich J., Lynam A., Miquelle D., Wibisono H., Kawanishi K., Pattanavibool A., Htun S., Tempa T., Karki J., Jhala Y., & Karanth U. (2015) Available at: www.iucnredlist.org.

Harris B. (2008) The potential impact of super-volcanic eruptions on the Earth's atmosphere. *Weather*, **63**, 221–225.

Harrison T. (1996) The palaeoecological context at Niah Cave, Sarawak: evidence from the primate fauna. *Bulletin of the Indo-Pacific Prehistory Association*, **14**, 90–100.

Hernandez P.A., Graham C.H., Master L.L., & Albert D.L. (2006) The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*, **5**, 773–785.

Herrington S.J. (1987) Subspecies and the Conservation of Panthera tigris: Preserving Genetic Heterogeneity. *Tigers of the World* (ed. by R.L. Tilson and U.S. Seal), pp. 51–61. Noyes Publications, Park Ridge, New Jersey.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., & Jarvis A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hooijer D.A. (1963) Further "Hell" mammals from Niah. *Sarawak Museum Journal*, **11**, 196–200.

Kaplan J.O., Bigelow N.H., Prentice I.C., Harrison S.P., Bartlein P.J., Christensen T.R., Cramer W., Matveyeva N.V., McGuire A.D., Murray D.F., Razzhivin V.Y., Smith B., Walker D.A., Anderson P.M., Andrew A.A., Brubaker L.B., Edwards M.E., & Lozhkin A.V. (2003) Climate change and Arctic ecosystems: 2. Modeling, paleodata-model comparisons, and future projections. *Journal of Geophysical Research*, **108**, 1–17.

Kitchener A.C. (1999) Tiger Distribution, Phenotypic Variation and Conservation Issues. *Riding the Tiger. Tiger Conservation in Human-Dominated Landscapes* (ed. by J. Seidensticker, J. Christie, and P. Jackson), pp. 19–39. Cambridge University Press, Cambridge.

Kitchener A.C. & Dugmore A.J. (2000) Biogeographical change in the tiger , Panthera tigris. *Animal Conservation*, **3**, 113–124.

Kitchener A.C. & Yamaguchi N. (2010) What is a tiger? Biogeography, morphology, and taxonomy. *Tigers of the World* (ed. by R. Tilson and P. Nyhus), pp. 53–84. Academic Press, London.

Kong F. (1992) The Destruction of Ancient Ecological Environment and the Movement of

Civilization Center in China. *Chinese Geographical Science*, **2**, 382–388.
Kozak K.H., Graham C.H., & Wiens J.J. (2008) Integrating GIS-based environmental data into evolutionary biology. *Trends in Ecology & Evolution*, **23**, 141–8.
Lorenzen E.D., Nogués-Bravo D., Orlando L., Weinstock J., Binladen J., Marske K. a, Ugan A., Borregaard M.K., Gilbert M.T.P., Nielsen R., Ho S.Y.W., Goebel T., Graf K.E., Byers D., Stenderup J.T., Rasmussen M., Campos P.F., Leonard J. a, Koepfli K.-P., Froese D., Zazula G., Stafford T.W., Aaris-Sørensen K., Batra P., Haywood A.M., Singarayer J.S., Valdes P.J., Boeskorov G., Burns J. a, Davydov S.P., Haile J., Jenkins D.L., Kosintsev P., Kuznetsova T., Lai X., Martin L.D., McDonald H.G., Mol D., Meldgaard M., Munch K., Stephan E., Sablin M., Sommer R.S., Sipko T., Scott E., Suchard M. a, Tikhonov A., Willerslev R., Wayne R.K., Cooper A., Hofreiter M., Sher A., Shapiro B., Rahbek C., & Willerslev E. (2011) Species-specific responses of Late Quaternary megafauna to climate and humans. *Nature*, **479**, 359–64.
Louys J. (2007) Limited effect of the Quaternary's largest super-eruption (Toba) on land mammals from Southeast Asia. *Quaternary Science Reviews*, **26**, 3108–3117.
Luo S.-J., Kim J.-H., Johnson W.E., van der Walt J., Martenson J., Yuhki N., Miquelle D.G., Uphyrkina O., Goodrich J.M., Quigley H.B., Tilson R., Brady G., Martelli P., Subramaniam V., McDougal C., Hean S., Huang S.-Q., Pan W., Karanth U.K., Sunquist M., Smith J.L.D., & O'Brien S.J. (2004) Phylogeography and genetic ancestry of tigers (Panthera tigris). *PLoS biology*, **2**, e442.
Luo S.-J., Zhang Y., Johnson W.E., Miao L., Martelli P., Antunes A., Smith J.L.D., & O'Brien S.J. (2014) Sympatric Asian felid phylogeography reveals a major Indochinese-Sundaic divergence. *Molecular ecology*, **23**, 2072–92.
Manamendra-Arachchi K., Pethiyagoda R., Dissanayake R., & Meegaskumbura M. (2005) A second extinct big cat from the late quaternary of Sri Lanka. *The Raffles Bulletin of Zoology*, 423–434.
Martínez-Meyer E., Peterson A.T., & Hargrove W.W. (2004) Ecological niches as stable distributional constraints on mammal species , with implications for Pleistocene extinctions and climate change projections for biodiversity. *Global Ecology and Biogeography*, **13**, 305–314.
Mazák J.H. (2010) Craniometric variation in the tiger (Panthera tigris): Implications for patterns of diversity, taxonomy and conservation. *Mammalian Biology - Zeitschrift für Säugetierkunde*, **75**, 45–68.
Mazák J.H., Christiansen P., & Kitchener A.C. (2011) Oldest Known Pantherine Skull and Evolution of the Tiger. *PloS one*, **6**, 1–11.
Mazák V.J. (1996) *Der Tiger.* Westarp Wissenschaften, Magdeburg.
Mazák V.J., Groves C.P., & van Bree P.J.. (1978) On a skin and skull of the Bali tiger, and a list of preserved specimens of Panthera tigris balica (Schwarz, 1912). *Zeitschrift für Säugetierkunde*, **43**, 108–113.
Meijaard E. (2004) Biogeographic history of the Javan leopard Panthera pardus based on a craniometric analysis. *Journal of Mammalogy*, **85**, 302–310.
Miehe G., Miehe S., Vogel J., Co S., & La D. (2007) Highest Treeline in the Northern Hemisphere Found in Southern Tibet. *Mountain Research and Development*, **27**, 169–173.
Mondol S., Bruford M.W., & Ramakrishnan U. (2013) Demographic loss, genetic structure and the conservation implications for Indian tigers. *Proceedings of the Royal Society B*, **280**, 1–10.
Muscarella R., Galante P.J., Soley-Guardia M., Boria R.A., Kass J.M., Uriarte M., & Anderson R.P. (2014) ENMeval: An R package for conducting spatially independent evaluations and estimating optimal model complexity for Maxent ecological niche

models. *Methods in Ecology and Evolution*, **5**, 1198–1205.
Nowell K. & Jackson P. (1996) *The Wildcats: A Status Survey and Conservation Action Plan.* IUCN, Gland, Switzerland.
O'Regan H.J., Turner A., & Wilkinson D.M. (2002) European quaternary refugia: a factor in large carnivore extinction? *Journal of Quaternary Science*, **17**, 789–795.
Oppenheimer C. (2002) Limited global change due to the largest known Quaternary eruption, Toba ~ 74 kyr BP? *Quaternary Science Reviews*, **21**, 1593–1609.
Park S.-C., Yoo D.-G., Lee C.-W., & Lee E.-I. (2000) Last glacial sea-level changes and paleogeography of the Korea (Tsushima) Strait. *Geo-Marine Letters*, **20**, 64–71.
Pearson R.G., Raxworthy C.J., Nakamura M., & Townsend Peterson A. (2006) Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography*, **34**, 102–117.
Phillips S.J., Anderson R.P., & Schapire R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.
Phillips S.J. & Dudík M. (2008) Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, **31**, 161–175.
Prothero D.R. (2004) Did impacts, volcanic eruptions, or climate change affect mammalian evolution? *Palaeogeography, Palaeoclimatology, Palaeoecology*, **214**, 283–294.
Radosavljevic A. & Anderson R.P. (2014) Making better Maxent models of species distributions: Complexity, overfitting and evaluation. *Journal of Biogeography*, **41**, 629–643.
Rampino M.R. & Self S. (1992) Volcanic winter and accelerated glaciation following the Toba super-eruption. *Nature*, **359**, 50–52.
Rebelo H., Froufe E., Brito J.C., Russo D., Cistrone L., Ferrand N., & Jones G. (2012) Postglacial colonization of Europe by the barbastelle bat: agreement between molecular data and past predictive modelling. *Molecular Ecology*, **21**, 2761–74.
Renner I.W., Elith J., Baddeley A., Fithian W., Hastie T., Phillips S.J., Popovic G., & Warton D.I. (2015) Point process models for presence-only analysis. *Methods in Ecology and Evolution*, **6**, 366–379.
Rohling E.J., Fenton M., Jorissen F.J., Bertrand P., Ganssen G., & Caulet J.P. (1998) Magnitudes of sea-level lowstands of the past 500,000 years. *Nature*, **394**, 162–165.
Rose W.I. & Chesner C.A. (1987) Dispersal of Ash in the Great Toba Eruption, 75 ka. *Geology*, **15**, 913–917.
Sangay T. & Wangchuk T. (2005) Tiger Action Plan for the Kingdom of Bhutan 2006-2015. .
Schickhoff U. (2005) The Upper Timberline in the Himalayas , Hindu Kush and Karakorum : a Review of Geographical and Ecological Aspects. *Mountain Ecosystems. Studies in Treeline Ecology* (ed. by G. Broll and B. Keplin), pp. 275–354. Springer, Berlin.
Shane P., Westgate J., Williams M., & Korisettar R. (1995) New Geochemical Evidence for the Youngest Toba Tuff in India. *Quaternary Research*, **44**, 200–204.
de Smith M.J., Goodchild M.F., & Longley P.A. (2006) *Geospatial Analysis.* Troubador Publishing Ltd, Leicester.
Sunquist M.E. (1981) The Social Organization of Tigers (Panthera Tigris) in Royal Chitawan National Park, Nepal. *Smithsonian Contributions to Zoology*, **336**, 1–98.
Tao W., Huijun W., & Dabang J. (2010) Mid-Holocene East Asian summer climate as simulated by the PMIP2 models. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **288**, 93–102.
Taylor K.E., Stouffer R.J., & Meehl G.A. (2012) An overview of CMIP5 and the experiment design. *Bulletin of the American Meteorological Society*, **93**, 485–498.
Team R.C. (2015) R: A language and environment for statistical computing. .
Varela S., Lobo J.M., Rodríguez J., & Batra P. (2010) Were the Late Pleistocene climatic

changes responsible for the disappearance of the European spotted hyena populations? Hindcasting a species geographic distribution across time. *Quaternary Science Reviews*, **29**, 2027–2035.

Veloz S.D. (2009) Spatially autocorrelated sampling falsely inflates measures of accuracy for presence-only niche models. *Journal of Biogeography*, **36**, 2290–2299.

Walston J., Robinson J.G., Bennett E.L., Breitenmoser U., da Fonseca G.A.B., Goodrich J., Gumal M., Hunter L., Johnson A., Karanth K.U., Leader-Williams N., Mackinnon K., Miquelle D., Pattanavibool A., Poole C., Rabinowitz A., Smith J.L.D., Stokes E.J., Stuart S.N., Vongkhamheng C., & Wibisono H. (2010) Bringing the tiger back from the brink-the six percent solution. *PLoS biology*, **8**, 6–9.

Waltari E., Hijmans R.J., Peterson A.T., Nyári A.S., Perkins S.L., & Guralnick R.P. (2007) Locating pleistocene refugia: comparing phylogeographic and ecological niche model predictions. *PloS one*, **2**, 1–11.

Whitmore T.C. (1984) *Tropical rain forests of the Far East.* Oxford University Press, Oxford.

Wilting A., Courtiol A., Christiansen P., Niedballa J., Scharf A.K., Orlando L., Balkenhol N., Hofer H., Kramer-Schadt S., Fickel J., & Kitchener A.C. (2015) Planning tiger recovery: Understanding intraspecific variation for effective conservation. *Science Advances*, **1**, 1–13.

Wong S. Te, Servheen C., Ambu L., & Norhayati A. (2005) Impacts of fruit production cycles on Malayan sun bears and bearded pigs in lowland tropical forest of Sabah, Malaysian Borneo. *Journal of Tropical Ecology*, **21**, 627.

Xue H.R., Yamaguchi N., Driscoll C.A., Han Y., Bar-Gal G.K., Zhuang Y., Mazak J.H., Macdonald D.W., O'Brien S.J., & Luo S.J. (2015) Genetic Ancestry of the Extinct Javan and Bali Tigers. *Journal of Heredity*, 1–11.

Yamaguchi N., Cooper A., Werdelin L., & Macdonald D.W. (2004) Evolution of the mane and group-living in the lion (Panthera leo): A review. *Journal of Zoology*, **263**, 329–342.

Chapter 4: A Kingdom in decline: Climate drives lion range contraction since c 21ka

Preface

This chapter builds upon the methodology outlined in *Chapter 3* to assess the degree of natural connectivity in the ranges of the lion within the Late Pleistocene and Holocene. This chapter has been submitted for review in *Diversity and Distributions* as a collaborative paper. The author contributions are detailed at the start of the Supplementary Information for this chapter.

Abstract

Ecological niche models and environmental stratification of palaeoclimate are used to reconstruct the changing range of the lion (*Panthera leo*) during the late Pleistocene and Holocene. The modern (early 21st century) range of the lion extends from southern Africa to the western Indian Subcontinent, yet through the 20th century it has been drastically reduced in extent and become increasingly fragmented as a result of human impacts. New, deeper time perspectives are added to these human impacts by assessing range shifts and population trends before current anthropogenic pressures developed. The results presented here show that lion habitat suitability has reduced throughout the Holocene, controlled by pluvial/interpluvial cycles. The aridification of the Sahara ~6ka dramatically reduced lion range throughout North Africa. The association of Saharan aridification with the development of pastoralism and the growth of sedentary communities, who practised animal husbandry, would have placed additional and lasting anthropogenic pressures on the lion. This research highlights the need to integrate the full effects of the Green Sahara into palaeoclimatic models, and provides a starting point for further continental-scale analyses of shifting faunal ranges through North Africa and the Near East during the Holocene. This scale of ecological niche modelling does not, however, explain the long-term genetic variation in the lion, and I conclude that narrow but substantial physical barriers such as rivers have likely played a major role in population vicariance throughout the Late Pleistocene.

Introduction

The overall aim of this chapter is to model the range changes of the lion (*Panthera leo*) driven by large-scale climatological transitions since the Last Glacial Maximum, and evaluate the likely consequences on population distribution and connectivity. This is the essential contextualisation for current and continuing anthropogenic impacts on the species. The known historical range of the lion included much of Africa and southeastern Europe; it extended to the Near East, the Arabian Peninsula, and southwest Asia as far as the Indian Subcontinent (Yamaguchi et al., 2004; Ray et al., 2005) but today this range is considerably reduced. This contraction is even more pronounced if the very closely related taxa, *P. (l.) spelaeus* (the Eurasian cave lion) and *P. (l.) atrox*, (the North American lion) are included. During the Pleistocene the combined mid-to-low latitude distribution of lions in general was almost ubiquitous except for hyper-arid desert and dense tropical rainforests (Barnett et al., 2009).

The lion is principally an inhabitant of woodland savannah, savannah and steppe grasslands, and the extent of these and other favourable habitats has varied through the Quaternary in response to climate change. Recent molecular studies recognise a deep division between the 'northern' lions (West Africa, Central Africa and North Africa/Asia), and 'southern' lions (North East Africa, East/Southern Africa and South West Africa) (Barnett et al., 2014; Bertola et al., 2016) with population divergence likely emerging since the last interglacial (120-140ka) (Bertola et al., 2016). Similar patterns are proposed for other savannah megafauna in Africa (Lorenzen et al., 2012; Bertola et al., 2016), suggesting a common environmental driver for genetic and population differentiation.

Whilst there is agreement on evidence for long-term genetic splits across the historical range of the lion, there is considerable variance in the proposed divergence times of lion populations as expressed both by the differences between studies, and through the credible confidence intervals stated through each analysis. The proposed causes of long-term genetic differentiation between populations are the bioclimatic conditions associated with pluvial (wetter) and interpluvial (drier) conditions of the Late Pleistocene, which caused widespread changes to preferred habitat, and affected the efficacy of potential geographical boundaries, such as large rivers (Lorenzen et al., 2012; Bertola et al., 2016). Similarly, the wider dispersal of the lion outside Africa has been attributed to changes in climate, with pluvial conditions in northern Africa and the Middle East around 60-47ka (Timmermann & Friedrich, 2016) being

thought to have enabled lion range expansion across Eurasia (Yamaguchi et al., 2004). Whilst lions are known to cross rivers, increasing water levels of tributaries of the Okovango River/Delta have been shown to affect crossing frequency in lions (Cozzi et al., 2013), and it is possible that the very large rivers of Africa have provided effective environmental barriers to lion dispersal, especially during the wetter conditions experienced in the Late Pleistocene. Conversely, river systems may have acted as pathways for dispersal and/or connection through arid areas by providing corridors of favourable habitat for both lions and their prey. Typically, however, confidence intervals on genetic divergence times are wide (Antunes et al., 2008; Barnett et al., 2014; Bertola et al., 2016), and thus direct correlations between them and known bioclimatic changes lack certainty.

Climate change has clearly driven major shifts in past distributions, and with the current pace and direction of global climate change, the lion faces the possibility of a catastrophic two-fold impact of interactions of different drivers. Over geological timescales, a species' changing range is a key dimension to interpreting its evolutionary history. It is important to assess the likely drivers of shifting geographical ranges, establish scales, directions and rates of change, and to examine currently fragmented, and recently extirpated populations. Expanding and contracting range shifts may have occurred through climatic and geographical changes, human influences or to changes in species assemblages. By associating the environmental tolerances of the lion with palaeoclimatic data, the role of a key driver of change can be established, provide likely scenarios for the timeframes of population separation or connection through periods of turbulent climatic conditions, and give critical contextualisation to future threats and conservation management of this vulnerable species.

In this chapter modelling is used to explicitly address previous biogeographical speculation of population connectivity and dispersal. The likely scales of climate-driven changes are assessed by modelling suitable lion habitat for key periods that exhibit the extremes of bioclimatic conditions within the Late Pleistocene and Holocene (Chevalier et al., 2017). An understanding of shifting lion ranges and lion population contiguity through this period is thereby constrained to within clearly defined limits. By so doing, a new deep-time perspective on the current deteriorating state of lion populations is provided. This is important for understanding the historical context of the species' present vulnerability, which could be further exacerbated by future global change. The lion is an iconic symbol of both Africa and India, but is suffering from rapidly declining numbers and geographical range mostly due to human activities (Bauer et al., 2016).

Methods

Here, the use Global Environmental Stratification (Metzger et al., 2013b) with ecological niche models is described to model environmental suitability of the lion under current and palaeoclimatic scenarios.

The ecological niches of large mammalian carnivores at continental scales are largely dependent on climate (Geffen et al., 2004; Varela et al., 2010), which has been used to model the ranges of big cats across Africa and Eurasia (Townsend Peterson et al., 2014; Cooper et al., 2016; Li et al., 2016). Mammal species are likely to have tracked consistent climate profiles since the LGM (Martínez-Meyer et al., 2004) and palaeoclimatic data are commonly used to infer mammal range shifts from previous glacial conditions to the present (Varela et al., 2010; Rebelo et al., 2012; Kohli et al., 2014; Cooper et al., 2016; Li et al., 2016). The climatic conditions of the Last Glacial Maximum (~21ka), mid-Holocene (~6ka) and present day capture the climatic extremes of the Late Pleistocene, encapsulating the variable degrees of contiguity and vicariance between populations over this period. The social structure of the lion may have influenced its ecological niche through prey preference and resource selection. While lion social group size and composition is flexible and will alter with habitat and prey population density (Bauer et al., 2003; Meena, 2009), fundamental switches from group to individual living are unlikely to have occurred over the time periods modelled within this study (Yamaguchi et al., 2004), thereby giving additional confidence in my approach. Within Africa, the role of any extant species in competitively excluding the lion from any otherwise bioclimatically suitable area is not considered, and no other large African mammalian carnivore has become extinct during the Late Pleistocene or Holocene (Faith, 2014).

The methods described here are presented graphically within *Supplementary Information S2*. A Maximum Entropy (MaxEnt) ecological niche modelling approach is used in conjunction with the production of Global Environmental Stratification Strata/Zones (Metzger et al., 2013b, 2013a; Soteriades et al., 2017) to explore the extent of lion distributions through the Late Pleistocene and Holocene under varying climatic fluctuations. Interpreting ecological niche modelling projections onto novel climates can be challenging due to the complexity of considering the effects of multiple input variables together. Therefore, the modelled lion range is examined in terms of Global Environmental Stratification Strata (GEnS) (Metzger et al., 2013b) to define the bioclimatic characteristics of suitable lion habitat.

Locality records were collected from across the known historical range, and from a range of independent sources so as to reduce the influence of sampling bias in the data (Fei & Yu, 2016) (*table 1*). The time period that these records were collected from represents the present climatic conditions of the lion, and enables the inclusion of records from now extinct populations. The recognition of spatial error in locality data is an important consideration (Raxworthy et al., 2007), but given the large home range sizes of lions, issues of locality error are mitigated when considering the maximum locality error of 50km.

Table 1: Source and number of locality points used in the ecological niche modelling process, compiled from new data (museum specimen locality descriptions) and from additional literature and resources.

Locality Source	**Number of localities**
iNaturalist grade GBIF Localities (iNaturalist, 2015)	781
Loveridge and Canney, 2009 (Loveridge & Canney, 2009)	134
Museum Record Descriptions	101
VertNet Records (Constable et al., 2010)	32
Black *et al*., 2013 (Black et al., 2013)	20
Barnett et al. 2014 (Barnett et al., 2014)	20
Black *et al*., 2013 (Black et al., 2013)	20
Banerjee and Jhala, 2012 (Banerjee & Jhala, 2012)	12
Cross *et al*., 2008 (Cross et al., 2009)	6
Total	**1135**

Four environmental variables are used in my analysis to represent dominant bioclimatic trends. The variables are Growing Degree-Days on a 0°C base (Metzger et al., 2013b), Temperature Seasonality (Hijmans et al., 2005), Aridity Index and Potential Evapotranspiration Seasonality (Zomer et al., 2007, 2008). These four variables show low correlation with each other and determine 99.9% of the total variation of 36 available bioclimatic variables (Metzger et al., 2013b). The modelling extent (-19°E, 94°W, - 36°S, 50°N) is defined by the area accessible to the lion over historical times.

The MaxEnt modelling approach was applied as outlined in Cooper *et al*., (2016) to create a habitat suitability model of the lion for the present day (see *Supplementary Information S3* for full parameters). Model performance was measured using the mean area under the receiver operator curve (AUC) (Phillips & Dudík, 2008) from k-fold cross-validation and spatially independent cross-validation using the ENMeval package (Muscarella et al., 2014) in R

(Team, 2015). Spatially independent cross validation is important given the potential for spatial autocorrelation of the localities. Threshold values of suitable/unsuitable area were derived from the MaxEnt model for comparison with global environmental strata. A modified lowest-presence threshold (Costa et al., 2010) was used to determine a binary output of suitable lion habitat. An omission error of 10% (e=10%) was used to determine this threshold, which accounts for a level of uncertainty in the quality of the locality records (Peterson et al., 2008).

Global Environmental Stratification (Metzger et al., 2013b) describes relatively similar biophysical environments, which are derived through statistical clustering of the principal components of the four bioclimatic variables described for my MaxEnt model. Using the modelling approach of Soteriades *et al*., (2017) the strata were created on a global scale for present-day conditions, and for the mid-Holocene and the Last Glacial Maximum coupled general circulation models, at a five-arc-minute resolution using the data-mining and machine-learning software Weka 3.6.4 (Frank et al., 2016). 125 derived strata were further aggregated into 18 easily interpretable, structured bioclimatic zones (Metzger et al., 2013b). Global Environmental Zones and Strata have been made available for present day, mid-Holocene and Last Glacial Maximum conditions at http://hdl.handle.net/10283/3274 (Cooper et al., 2019).

The area of suitable habitat dictated by the modelled threshold was calculated for each environmental strata and zone, as was the total extent used in modelling. Strata were categorised as highly favoured, favoured, utilised, low use and unsuitable, where modelled threshold suitable habitat accounts for 80-100% (highly favoured), 60-80% (favoured), 40-60% (utilised), 10-40% (low use) and <10% (unsuitable) of the total modelling extent. These categories were then expressed on the strata for the present-day, mid-Holocene and Last Glacial Maximum scenarios. The modal value of suitability is displayed for the multiple mid-Holocene and LGM models (or values if two similar suitabilities cause a split agreement, e.g. favourable/highly favourable). If model results have no agreement, or the agreement is split between very different suitabilities, e.g. favourable/unsuitable, the strata were categorised as uncertain.

The area of each Global Environmental Zone within the IUCN Red List's extant lion distribution data was calculated to compare the modelled fundamental niche with the realised niche of current lion range. To provide environmental context to Global Environmental

Zones and Strata within the modelling extent, the proportion of MODIS land cover classes represented by each Zone and Strata was calculated.

Results

Lion environmental preferences

The quantified climatic preferences of the lion using a MaxEnt ecological niche model of habitat suitability are shown in *Figure 1*. The AUC value from model 10-fold cross validation was 0.923. The model AUC score from spatially independent cross-validation, using the 'checkerboard2' method (Muscarella et al., 2014), was 0.818. The modified lowest-presence threshold (e=10%) derived from the MaxEnt model was used to calculate 'highly favoured', 'favoured', 'utilised', 'low use' and 'unsuitable' Global Environmental Strata and Zones within the modelling extent (*Figure 2, Figure 3*). Highly favoured and favoured lion habitats predominantly consist of hot and mesic, hot and dry, extremely hot and xeric, and extremely hot and moist environmental zones (*Figure 2*). The modelled scenarios show a wider present day habitat tolerance than current known lion distributions derived from IUCN data (Bauer et al., 2016), with some favourability modelled within warm temperate zones (*Figure 2*). Within preferred environmental zones, some strata are low use or unsuitable. Each environmental stratum is compared to MODIS (Friedl et al., 2010; Channan et al., 2014) land cover classes to gain insight into the typical land covers of each stratum (see *Supplementary Information S6*). Today's lions prefer strata which are typified by woody savannahs and savannahs, rather than barren/sparse cover or more closed forest covers, which can occur within the same broader environmental zones.

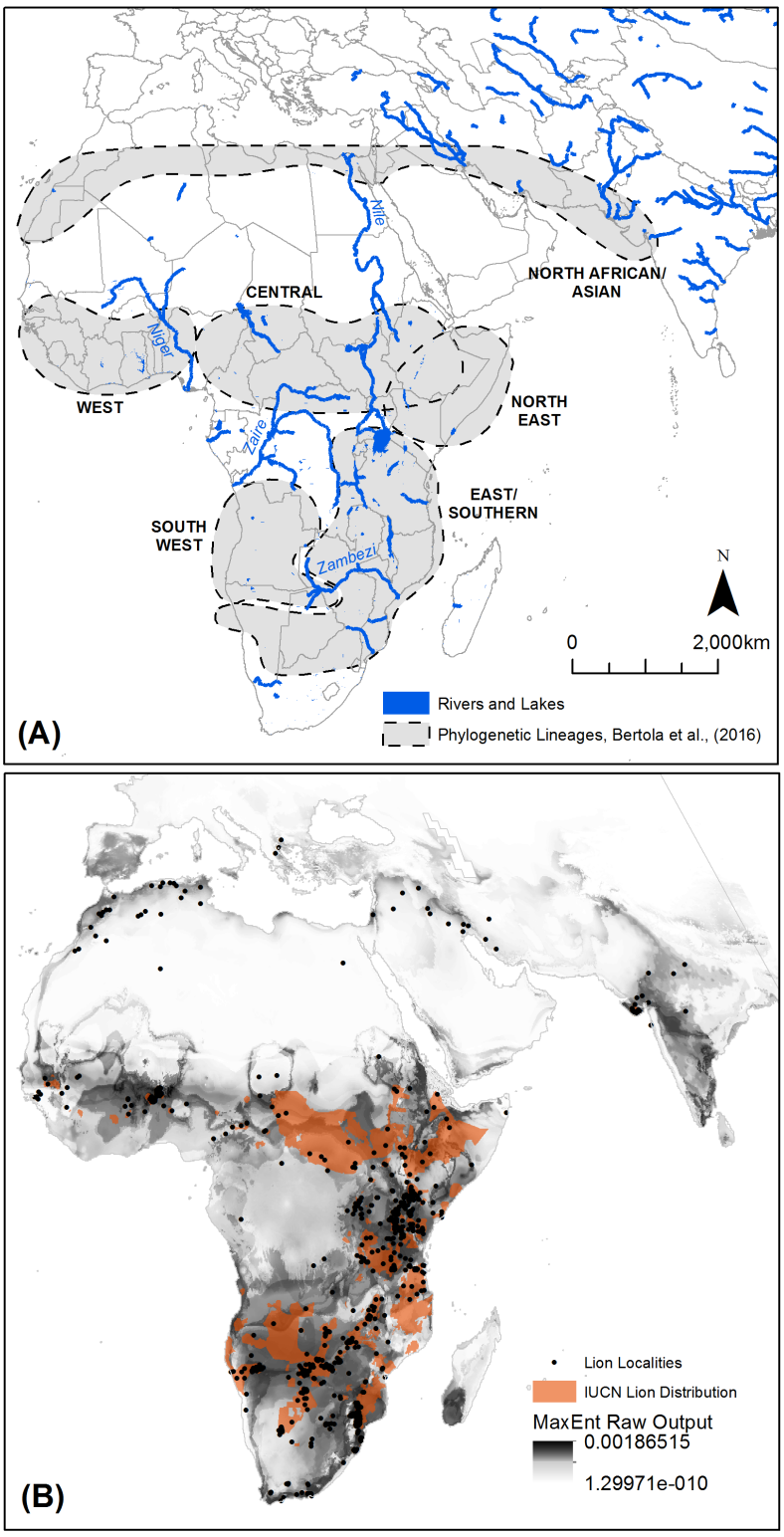


Figure 1: The proposed genetic demarcations of the modern lion (Bertola et al., 2016) and the location of large rivers and lakes as potential influencers of lion dispersal that are not accounted for in the models are highlighted **(A)**. The raw output of the MaxEnt model displays areas climatically favourable to the lion **(B)**. The current known range of the modern lion (orange) is restricted to a subset of modelled favourable habitat.

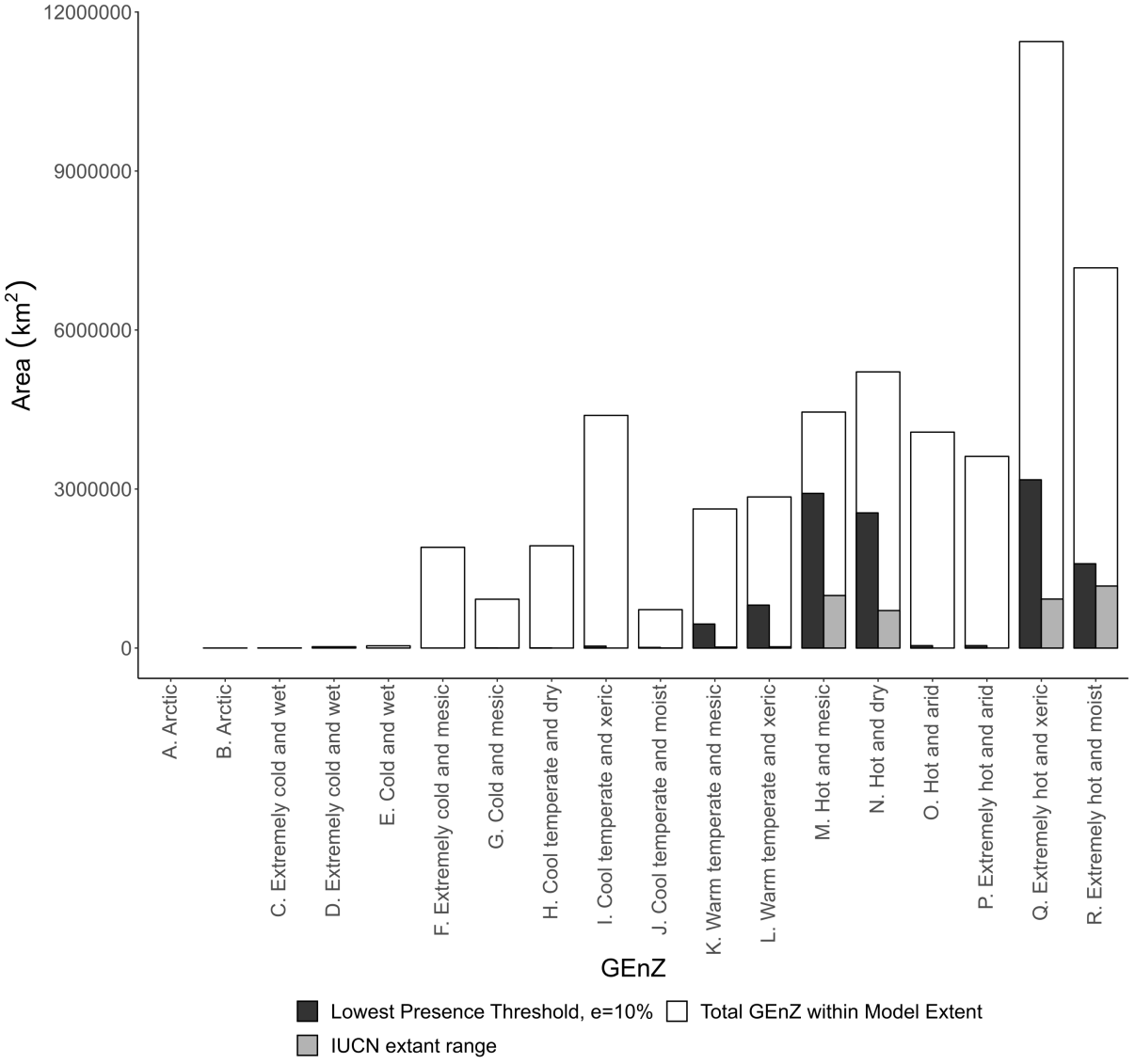


Figure 2: The proportion of Global Environmental Zones that is occupied by modelled lion distribution based on climatic suitability, and on IUCN extant lion range within the modelling extent. Lions occupy warm temperate and mesic, hot and mesic, hot and dry, extremely hot and xeric, and extremely hot and moist habitat as shown by both modelled results and extant distributions. Hot and mesic, and hot and dry habitats are particularly favoured under idealised model scenarios. Significant reductions in extant range, compared with modelled range, were likely to be caused by anthropogenic pressures.

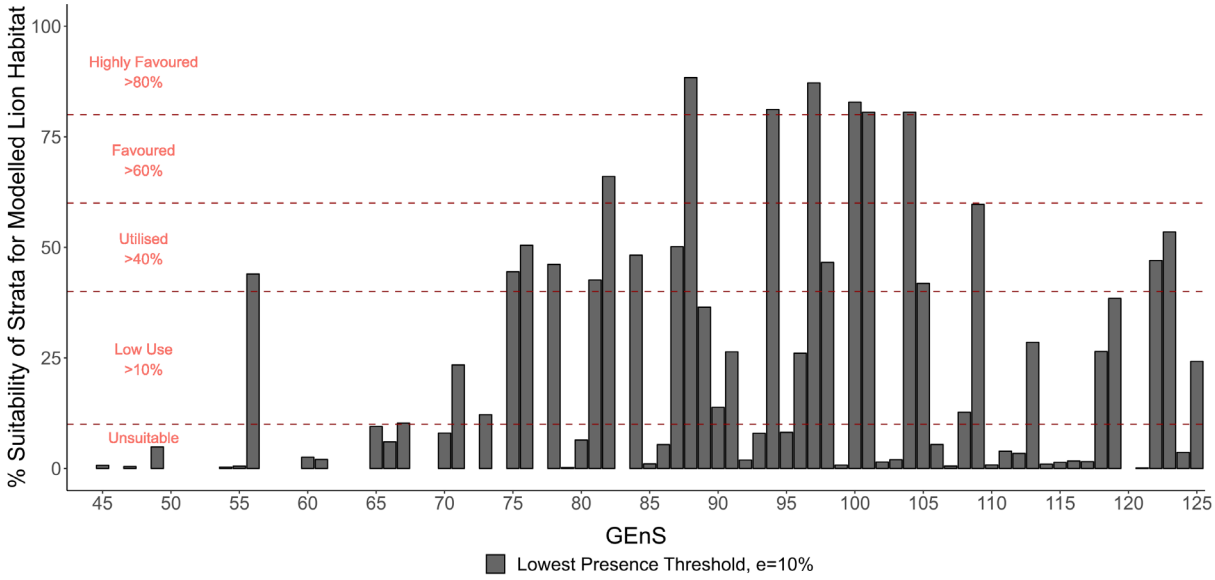


Figure 3: The percentage of Global Environmental Strata occupied by the modelled lion distribution within the modelling extent. This was used to inform maps of favourable Global Environmental Strata for the lion (Figure 4).

Modelled Holocene environmental suitability for the lion

The preferred lion habitat is projected upon modelled global environmental strata for present-day conditions, the mid-Holocene ~6ka and Last Glacial Maximum (LGM) ~21ka (*Figure 4*). Mid-Holocene and LGM outputs were derived from multiple coupled general circulation models (see *Supplementary Information S4*). Suitable conditions for the lion have fluctuated considerably since the LGM. Compared with present-day interglacial/interpluvial conditions, the LGM was considerably more favourable for the lion in both overall 'favourable' environmental conditions, and connectivity across the historical range. Favourable conditions across models consistently reduced for the Indian Subcontinent from the LGM, through the mid-Holocene and into present-day conditions. *Figure 5* highlights the reduction of highly favoured, favoured and utilised strata and increases in unsuitable and low use strata from the LGM to the present. Whilst LGM conditions are more favourable to the lion than modelled mid-Holocene or present-day conditions, the core 'favourable' environment has markedly shifted. During the LGM, the favourable lion habitat is consistently modelled across the Sudanian region with comparatively less favourable conditions than the mid-Holocene and present-day in southern Africa. The modelled results show the LGM as the most likely time for dispersal out of Africa to the Near East and Indian Subcontinent, but the extent and quality of the linkage is low. In all timeframes, and all modelling scenarios, there is little suitable habitat modelled within the Near East, and modelled suitability in southern Europe

has a small geographical range. The Congo basin has progressively become less favourable to the lion since the LGM.

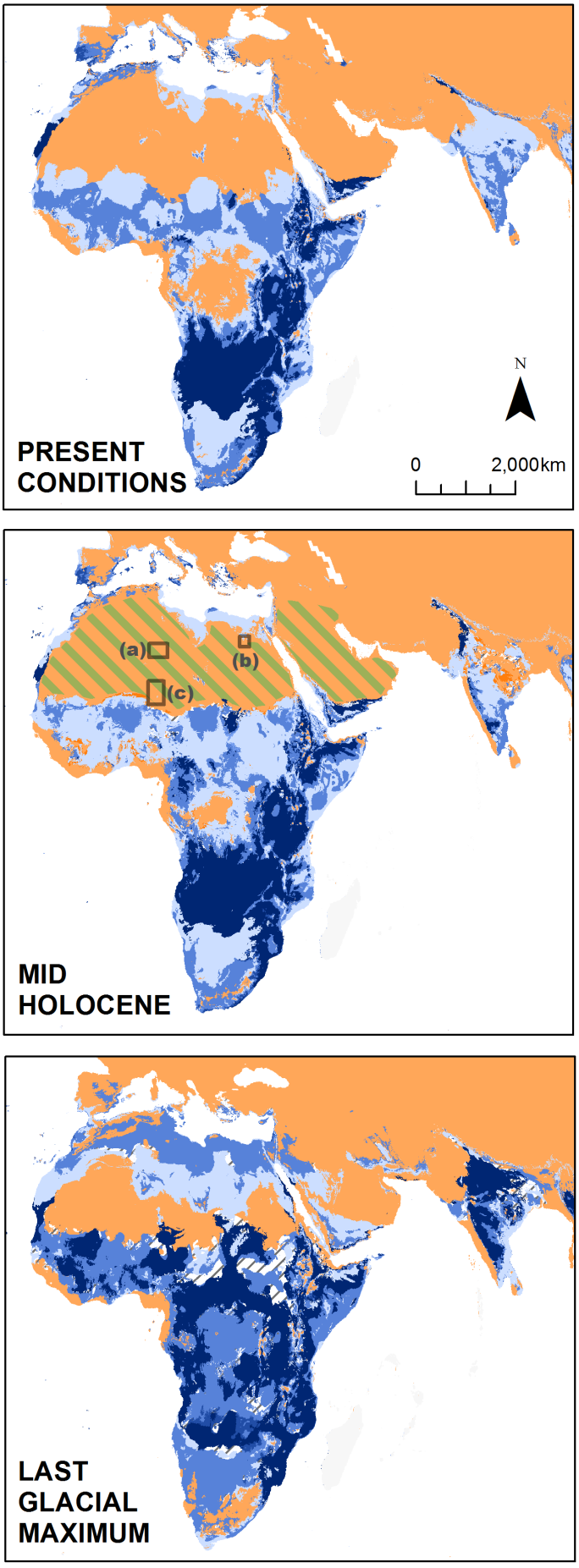


Figure 4: Modelled lion habitat suitability for the present day, mid-Holocene and Last Glacial Maximum, based on global environmental strata (GEnS). Mid-Holocene and Last Glacial Maximum maps represent the combined suitability based upon nine and three coupled general circulation models respectively. An area of uncertainty is included surrounding the mid-Holocene greening of the Sahara and Arabia (Hoelzmann et al., 1998; Larrasoaña et al., 2013) and evidence of Lions and other savannah megafauna at (a) Tassili n'Ajer, (b) Wadi el-Obeid and (c) Aïr (Galvin, 2018) (see *Supplementary Information S5* for records).

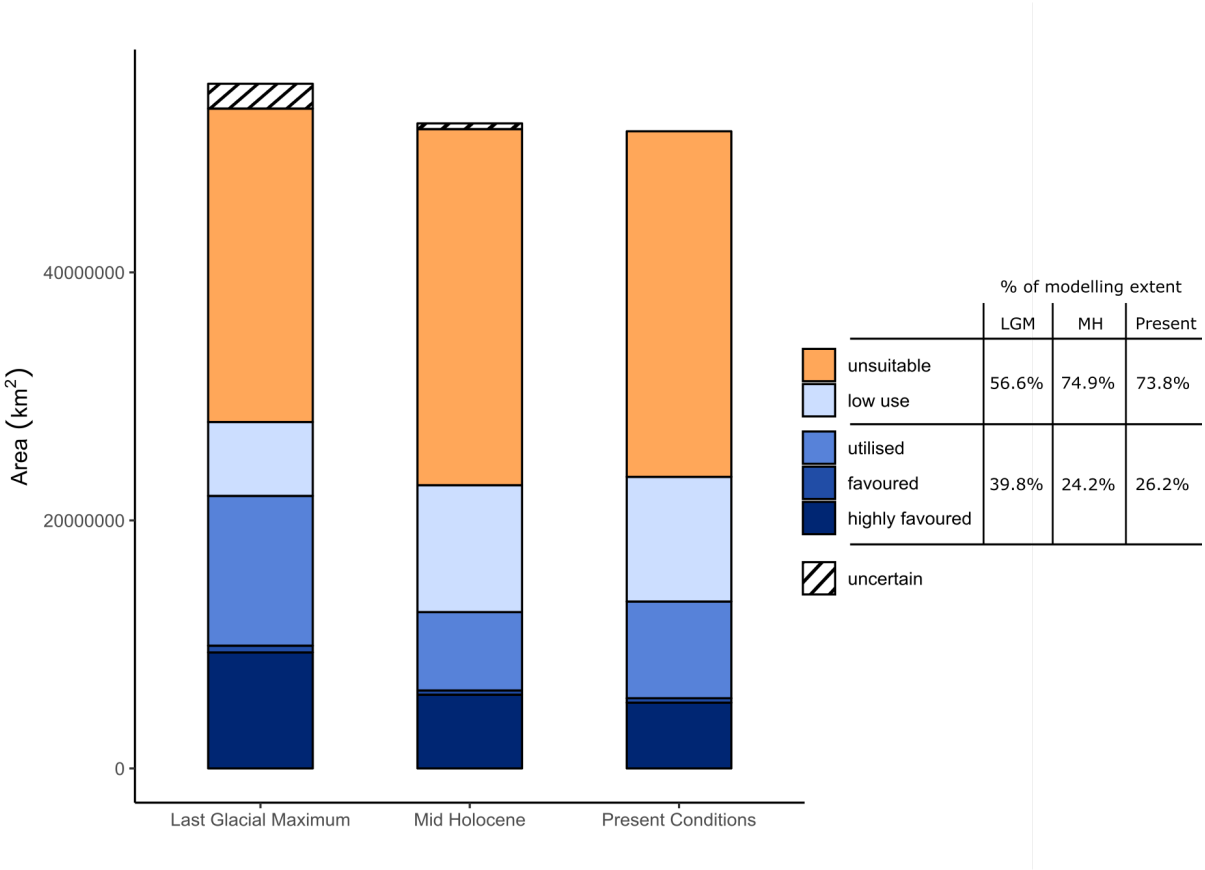


Figure 5: The changing area of favourable climatic conditions is shown for the present day, the mid-Holocene and the Last Glacial Maximum. Where model uncertainty exists between underlying mid-Holocene projections, the classes "highly favoured/favoured", "favoured/utilised", "low use/utilised" and "unsuitable/utilised" are collapsed into "highly favoured", "favoured", "utilised" and "low use" respectively. Utilised, favoured and highly favoured strata are more prevalent during the Last Glacial Maximum (39.8% of total area) than for either the Mid-Holocene (24.2%) or present day (26.2%), which are characterised by greater areas of unsuitable and low use strata. The total area for the LGM is greater than the present day and mid-Holocene due to lower sea levels at this time.

Discussion

Climate stratification has been used in the creation of biological monitoring programmes, including the construction of sampling strategies for species distribution models (Metzger et al., 2013a), but it has seen very limited application to the evaluation of these models or to mapping past faunal ranges (Hickie, 2016). Whilst the use of Global Environmental Stratification to create maps of suitability is visually similar to the underlying raw MaxEnt models, it also permits more in-depth analysis of preferred lion habitat, within a general

descriptive framework that can be extended to other species, time periods and geographical locations.

The mapping of lion habitat suitability in terms of Global Environmental Strata, provides an insight into their preferred Global Environmental Zones. Whilst certain environmental zones are more favoured by lions, no zone is modelled as universally suitable for the lion, as both favoured and highly favoured strata are found within environmental zones that include unsuitable and low-use strata. This is probably due to the lions' wide habitat tolerance within transitional landscapes, but limited tolerance of climatic extremes. Lions are found from semi-desert to dense woodland, with highest population densities found within moist savannah (Celesia et al., 2010) - lion distributions can be expected within semi-desert, but not true desert (Extremely hot and xeric), and in tropical forest, but not dense rainforest (Extremely hot and moist). The non-linear nature of vegetation cover through climatic gradients (Scheffer et al., 2012) has likely also played a role in the complex suitability of each environmental zone, as highlighted in the association between environmental strata and MODIS landcover (see *Supplementary Information S6*)

The underlying GEnS/Z datasets created here suggest that whilst increased vegetation may have penetrated desert zones up to 500km northwards of today's southern limits, (as reported by Willis *et al*., (2013)), inhospitable, hot and arid and extremely hot and arid climates persisted through much of the Sahara during the mid-Holocene (*Figure 6*). Crucially, however, this persistence of hot and arid conditions is not consistent with a wide body of evidence suggesting that large parts of the Sahara were characterised by well-connected (mega) lakes, rivers and inland deltas during the African Humid Period ~11-4ka (Hoelzmann et al., 1998; Drake et al., 2011; Migliore et al., 2013; Willis et al., 2013), and populated by a diverse assemblage of present-day sub-Saharan megafauna (Yeakel et al., 2014). In the absence of a comprehensive fossil record, the locations of African rock art which depict lions and other large megafauna, from within the African Humid Period ~11-5ka are included (*Figure 4, see table S7 for references*). This supports a wider distribution of lions than suggested through the modelling presented here. There is a strong argument that during the 'Green Sahara' episode, the Arabian Peninsula through to the western Indian Subcontinent also experienced wetter conditions, as these were affected by the same monsoonal forcing (Hoelzmann et al., 1998; Jones et al., 2013; Jennings et al., 2015). The disparity between GEnS/Z datasets and other palaeoenvironmental evidence exists because of the short-comings

of the underlying palaeoclimatic data within the Saharo-Arabian Region which has driven my models. The PMIP3 experiments, which drive the WorldClim bioclimatic datasets in my models, do not reproduce the Green Sahara, because changes in desert dust are not accounted for, and the vegetation feedbacks are either weak or non-existent (Tierney et al., 2017). As a result, the modelling here is likely to have only captured a minimum distribution of lions within the Sahara during the mid-Holocene. In reality it is likely that during African Humid Period suitable lion habitat was far more extensive across the Sahara and Arabia, and probably southwest Asia as well. Given the overall weight of evidence, it is likely that during the African Humid Period the series of connected rivers, lakes and deltas existed across the Sahara (Drake et al., 2011) . The ecological changes related to this altered hydrology would have either facilitated the movement of lions across the region by creating favourable lakeshore and riparian habitat corridors, or constrained their dispersal by creating water barriers to movement. Thus, the recognition (and confirmation) of a 'Green Sahara' has significant implications for a more detailed understanding of long-term variations of lion population size, and patterns of dispersal within and out of North Africa and the Near East.

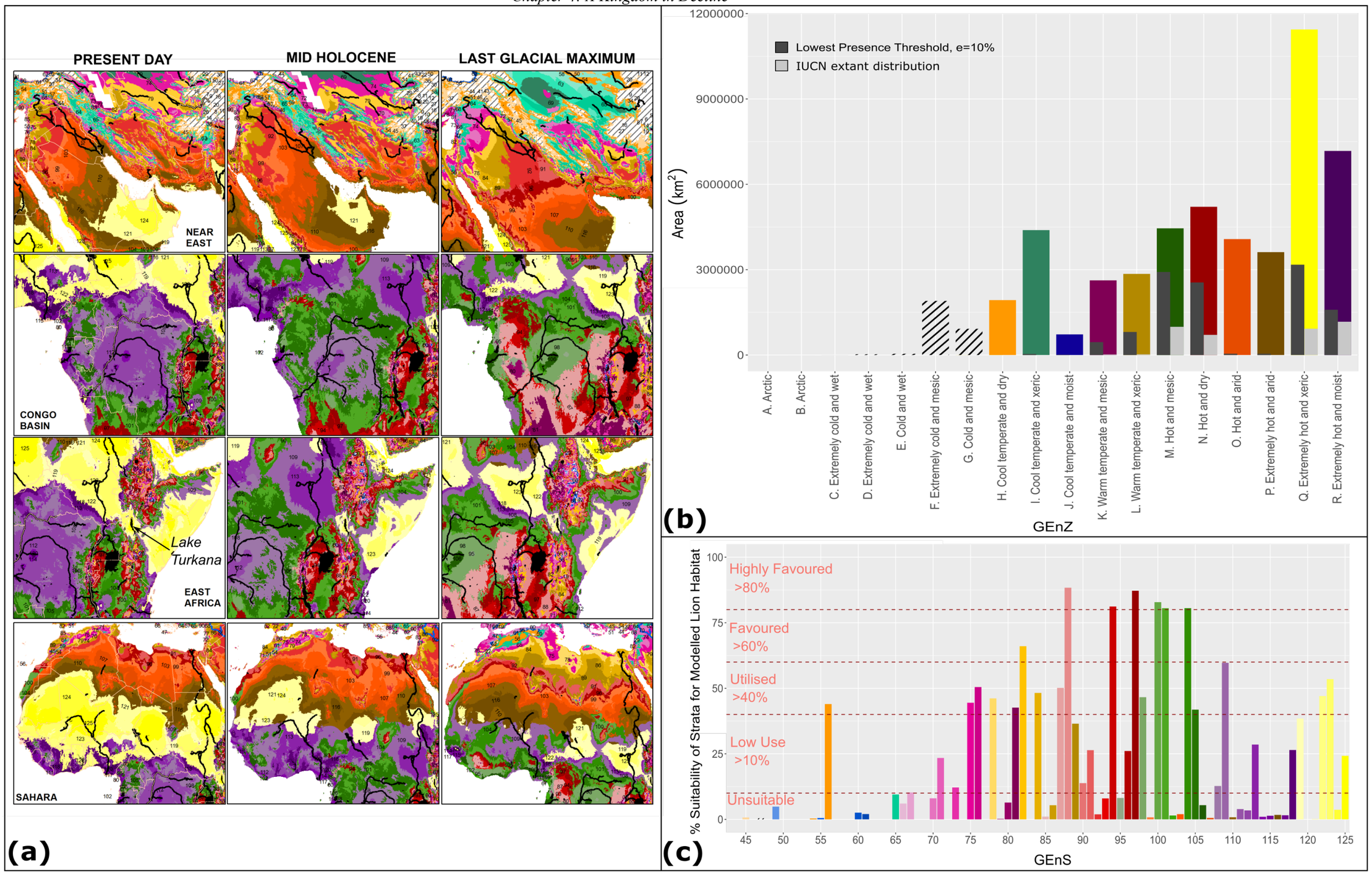


Figure 6: Global Environmental Strata (GEnS) are displayed for key areas across present, mid-Holocene and Last Glacial Maximum conditions (a). Strata colours are grouped into shades corresponding to Global Environmental Zone (b) – here, the total area of each Global Environmental Zone within the modelling extent, and the suitability of each zone for the lion based upon the models, and on current lion range determined by the IUCN is displayed. The colours of each strata (a) are matched to the associated bar chart of Global Environmental Strata (c), which displays the suitability of each strata for the lion based upon the MaxEnt ecological niche model.

There is good evidence of lion presence within the Near East and southeastern Europe into historical times (Bartosiewicz, 2009; Schnitzler, 2011), yet the modelling here shows that today these regions have a particularly poor climatic suitability for lions. One explanation for this could be lion survival in inter-pluvial refugia formed around river systems and water points, and the endurance of relict populations from a previous contiguous range (Black et al., 2013).

Modelling of the LGM shows some limited climatic suitability for lion dispersal between Africa and the Indian Subcontinent. This potential is most pronounced south of the present-day An Nafud desert, through the northern Persian Gulf, and eastward through the southern Zagros Mountains and Balochistan. This corridor is characterised by warm temperate and xeric, warm temperate and mesic, and hot and dry environmental zones, and strong environmental gradients across the strata (*Figure 5*). It has been assumed that the lion moved out of Africa via the Sinai Peninsula (Barnett et al., 2014), but the potential dispersal of Hamadryas baboons, *Papio hamadryas*, to Arabia via the Bab-el-Mandab during the Late Pleistocene (Kopp et al., 2014), when sea levels were lower, raises the possibility that this route was also used by the lion. Whilst lions may have crossed the narrow strait to small areas of favourable habitat, this analysis indicates a parallel and better supported dispersal could have occurred from the Sinai Peninsula into the Arabian Peninsula. Pluvial conditions during the mid-Holocene Wet Phase, whilst not explicitly modelled, were probably associated with more rainfall than the LGM (Tierney et al., 2017) and would have provided even more favourable conditions for dispersal and associated gene flow through North Africa and the Near East. Individual lions are highly mobile, with individual males known to disperse >200km within 1-2 years (Funston et al., 2003), and even fleeting favourable conditions would have created opportunities for genetic flow.

I argue that recent historical populations in the Near East are not an indication of long-distance dispersal routes given the presence of climatic barriers and lack of continuous riparian corridors. About 6000 years ago, the latest phase of aridification across the Sahara and Arabia probably separated lions in India from those in Africa. The Gir population appears to be a relict of more favourable palaeoclimatic conditions, but there are still significant areas within the Indian Subcontinent which appear to be climatically favourable today, such areas of the Deccan Plateau east of the Western Ghats. Within this eastern range,

potential ecological competition with the sympatric tiger, *Panthera tigris*, may also constrain the lion's potential present-day distribution.

The modelling presented here indicates that through the pluvial/interpluvial cycles of the Holocene the sub-Saharan range of lions has always been contiguous, and so there is no evidence for any significant gaps between populations caused by depopulated zones of climatically unfavourable terrestrial habitat. This implies that something else has created barriers to lion dispersal between Western African and Eastern/Southern African populations. This is significant given the pre-Holocene genetic divergence amongst these lion populations (Antunes et al., 2008; Barnett et al., 2014; Bertola et al., 2016), and numerous other large mammalian grassland/savannah species (Bertola et al., 2016). Thus, patterns of regional climate alone are not able to explain longer-term genetic divergence between populations. A combination of less favourable climatic conditions surrounding Lake Turkana within Africa's Rift Valley (*Figure 6*), and the presence of major physical barriers such as Lake Turkana itself, other Rift Valley lakes, the Omo River and Nile River systems (*Figure 6*), may have significantly reduced gene flow for large, mobile mammal species such as the lion. The contiguous favourable conditions found in eastern southern Africa throughout the changing climatic conditions of the Late Pleistocene supports the argument for this region as the evolutionary cradle of the modern lion (Barnett et al., 2014). Given the identified discrepancies between the coupled General Circulation Models and climatic proxy data in the northern hemisphere, it is possible that model inconsistencies exist within other regions of interest. PMIP3 experiments show good agreement with palaeoclimate proxy data for the mid-Holocene within eastern Africa, and this region was likely wetter than is modelled during the LGM, whilst south Eastern Africa was likely drier and cooler (Barker & Gasse, 2003; Chevalier et al., 2017), with African rift lake levels similar to those found today (Barker & Gasse, 2003). However, the impact of these differences during the LGM is not of the same scale or extent as those found within the Sahara and Arabia during the mid-Holocene. The lowest agreements between PMIP3 models occur towards coastal regions, and differences are not homogenous across East Africa (Singarayer & Burrough, 2015). However, it is possible that different conditions during the LGM within eastern Africa could have affected lion distributions, and therefore could have contributed to the long-term vicariance of northern and southern populations.

The wetter conditions across North Africa ('Green Sahara') might have effectively counteracted the range reduction in other areas since the LGM and would have allowed lion expansion from southern Africa to northern Africa, although widespread mixing of populations is not supported by current genetic evidence except in northeast Africa. In recent historical times the centre of gravity of lion distribution has lain in southern or eastern Africa, but in the past it could have been much further north. Following the desertification of the Sahara, the range changes in the north are proportionally far greater than those in the south, and this may have led to increased genetic drift through population isolation (Yamaguchi et al., 2004). The reduction in suitable habitat for the lion through the Holocene, and especially following the aridification of the Sahara and Arabia has coincided with the development of agricultural systems and rising anthropogenic pressures, creating a double impact on the lion. This modelled range change is consistent with previous skyline plots of lion populations which show a recent precipitous drop (Bertola et al., 2016). The position of major rivers through Africa, in addition to contractions of suitable habitat driven by climate change, are the likely causes of vicariance over 100ka. Suture zones and parapatric (sub)speciation are likely important in maintaining genetic variation. The persistence of major river barriers/corridors in the region, appears to have major biogeographical legacies in terms of defining boundaries between populations and linkage between areas.

The long-term trend in lion range reduction from the LGM into present-day conditions as revealed by the modelling becomes even more pronounced if we consider the possible extent of more benign conditions across the Sahara and Arabia during the African Humid Period and the subsequent persistence of hyper-arid conditions through the region after ~4.3ka (Kröpelin et al., 2008). A rapid decline in overall lion numbers as a result of mid-Holocene range contractions driven by climate change is compatible with a population skyline plot derived from genetic analysis (Bertola et al., 2016). Although the ranges of lions south of the Sahara remained contiguous, climatic change may have led to poor connectivity amongst West African, North African, and European/Asian lion populations. In addition to climate change, the Holocene has witnessed an increasing human impact on lions because humans and lions flourish in the same areas (Kuper & Kröpelin, 2006), and ultimately competition for favoured habitats has driven the anthropogenic pressure on lions today. Conflicts are likely to have arisen alongside domestication and the development of pastoralism as lions would prove a significant threat through predation of cattle and other livestock. Short-lived aridification of the Sahara ~8ka is associated with widespread transition to pastoralism from hunter-gathering

(Dunne et al., 2012; Tierney et al., 2017). With increased aridification, human populations congregated with their domestic livestock within the same ecological refuges as lions (Kuper & Kröpelin, 2006), thus exacerbating direct conflict between people and lions that probably drove the local extinctions of lions, and created new barriers to lion dispersal and gene flow.

In conclusion, there has been a long-term reduction of lion numbers during the Holocene, driven by the coincidental and combined influence of climate change and human impacts. The significant and continuing reduction in lion numbers during the 20th/21st centuries (Black et al., 2013; Bauer et al., 2016) is occurring in the context of a global population under increasing pressure. A mutually reinforcing effect of range reductions driven by climate and an intensification of human predation, as found during the last ~6ka, is likely to further intensify in the future.

Core findings

- There has been a general reduction in lion range from the LGM, through the mid-Holocene to the present day.

- Modelling indicates that lion ranges south of the Sahara have been contiguous, so climatic drivers are not responsible for long-term vicariance in lion populations, which could instead be the result of the discrete geographical barriers formed by rivers, lakes, mountains, etc.

- Modelling does not identify any significant areas of favourable habitat for lions extending across the Zagros mountains or the Tigris-Euphrates river basin. As a result no obvious climatic explanation for lion expansion out of Africa is identified; although this is likely explained by poor model performance within North Africa and the Near East during the mid-Holocene.

- The presence of a Green Sahara is not apparent in the Environmental Zones/Strata, as expected due to the shortfalls of PMIP3 simulations, and this limits our understanding of lion distributions in North Africa during the mid-Holocene.

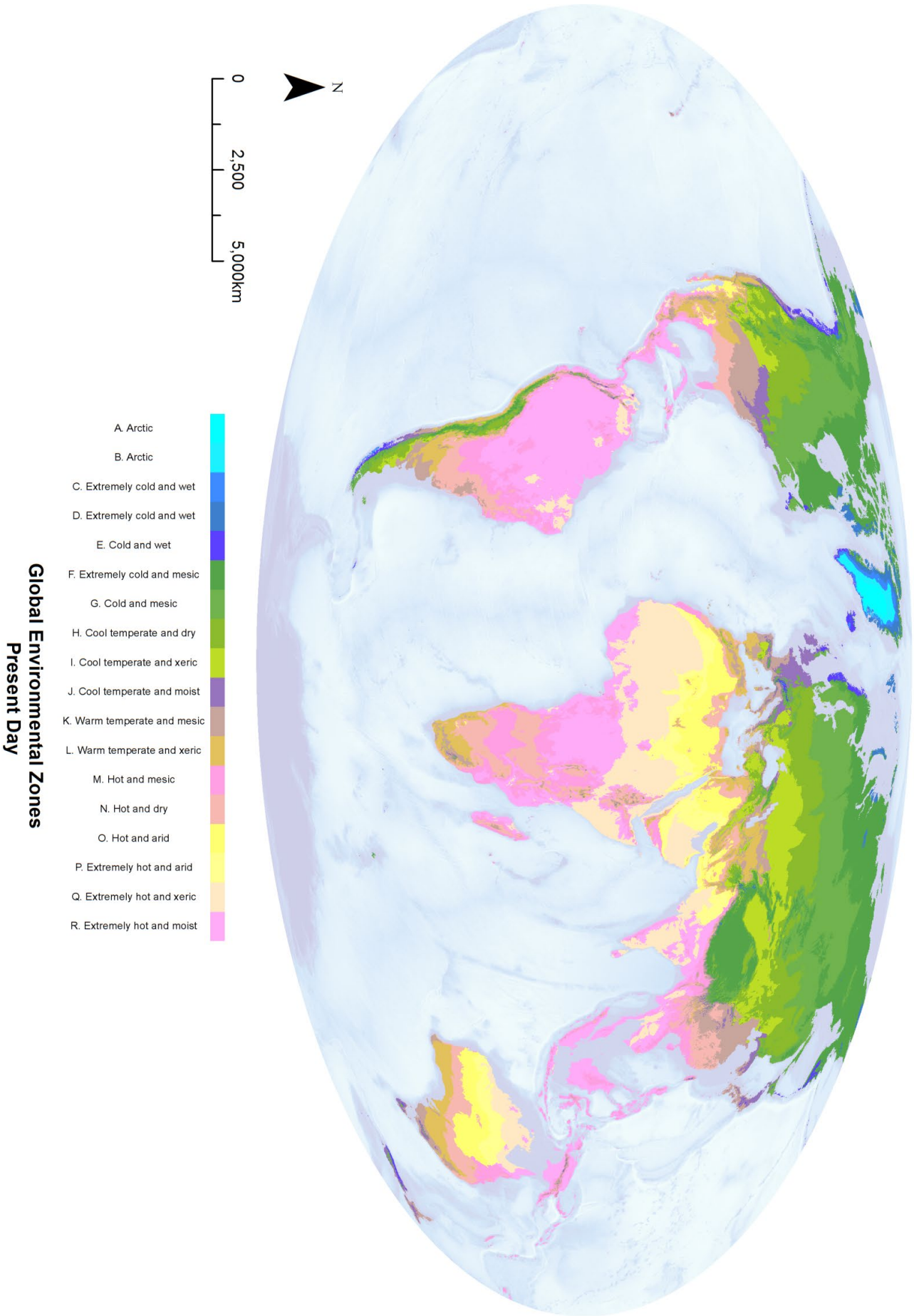
- Global Environmental Stratification provides a modelling framework that facilitates descriptive interrogation of my findings.

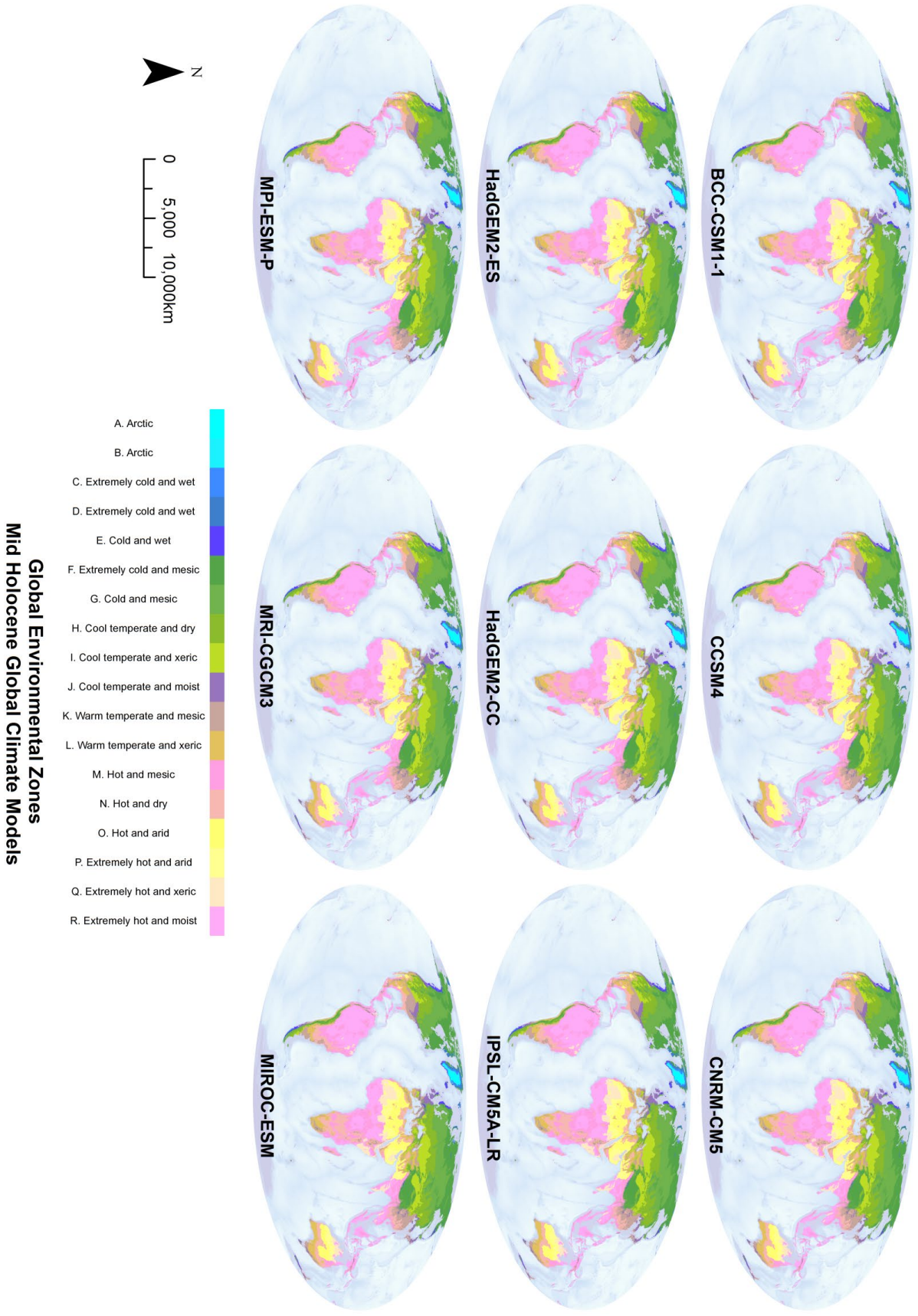
Supplementary Information

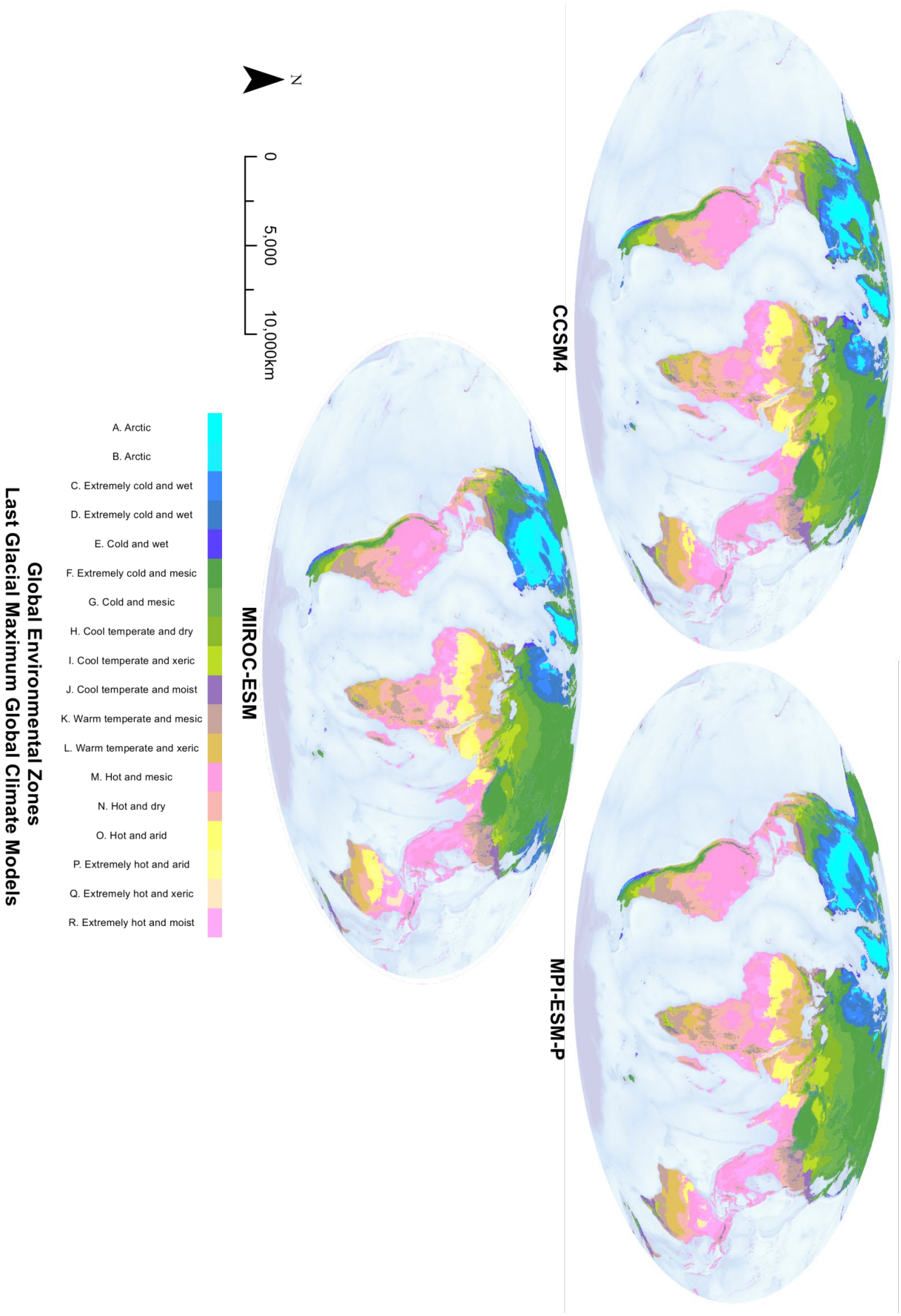
Author contributions

This chapter has been submitted to *Diversity and Distributions* as part of a collaborative paper with myself as lead author. The data was collated, analysis performed and writing conducted by myself. Andrew Dugmore, Andrew Kitchener and Marc Metzger helped design the research. Antonio Trabucco provided raw bioclimatic data.

Appendix S1: High resolution figures of Global Environmental Zones for the present day, mid-Holocene, and Last Glacial Maximum


Global Environmental Zones
Present Day
A. Arctic
B. Arctic
C. Extremely cold and wet
D. Extremely cold and wet
E. Cold and wet
F. Extremely cold and mesic
G. Cold and mesic
H. Cool temperate and dry
I. Cool temperate and xeric
J. Cool temperate and moist
K. Warm temperate and mesic
L. Warm temperate and xeric
M. Hot and mesic
N. Hot and dry
O. Hot and arid
P. Extremely hot and arid
Q. Extremely hot and xeric
R. Extremely hot and moist
0
2,500
5,000km
N

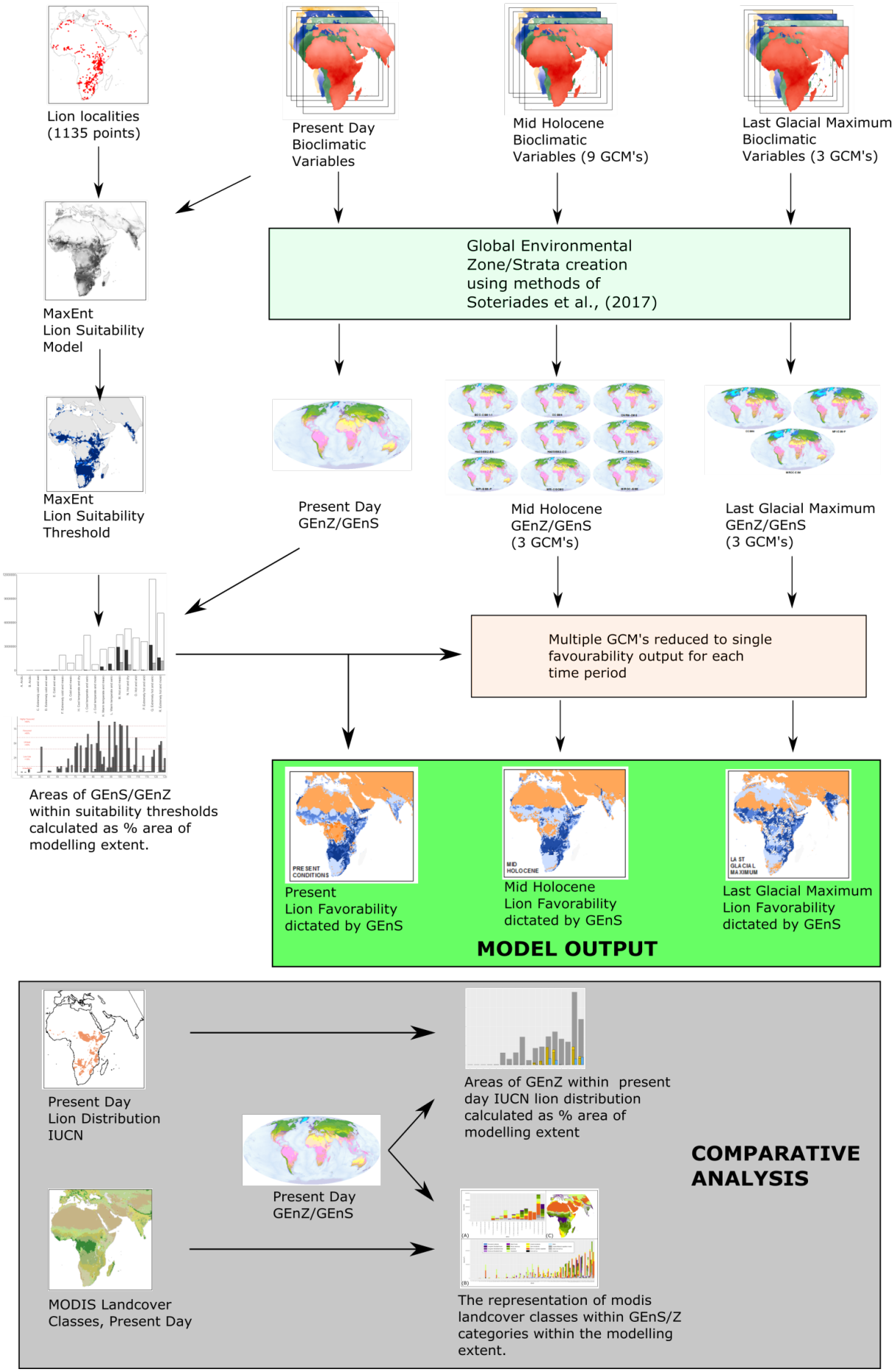

Global Environmental Zones
Mid Holocene Global Climate Models
A. Arctic
B. Arctic
C. Extremely cold and wet
D. Extremely cold and wet
E. Cold and wet
F. Extremely cold and mesic
G. Cold and mesic
H. Cool temperate and dry
I. Cool temperate and xeric
J. Cool temperate and moist
K. Warm temperate and mesic
L. Warm temperate and xeric
M. Hot and mesic
N. Hot and dry
O. Hot and arid
P. Extremely hot and arid
Q. Extremely hot and xeric
R. Extremely hot and moist
N
0 5,000 10,000km
MPI-ESM-P
HadGEM2-ES
BCC-CSM1-1
MRI-CGCM3
HadGEM2-CC
CCSM4
MIROC-ESM
IPSL-CM5A-LR
CNRM-CM5


Global Environmental Zones
Last Glacial Maximum Global Climate Models
A. Arctic
B. Arctic
C. Extremely cold and wet
D. Extremely cold and wet
E. Cold and wet
F. Extremely cold and mesic
G. Cold and mesic
H. Cool temperate and dry
I. Cool temperate and xeric
J. Cool temperate and moist
K. Warm temperate and mesic
L. Warm temperate and xeric
M. Hot and mesic
N. Hot and dry
O. Hot and arid
P. Extremely hot and arid
Q. Extremely hot and xeric
R. Extremely hot and moist
0
5,000
10,000km
N
CCSM4
MIROC-ESM
MPI-ESM-P

Appendix S2: Methodological Overview

Methodology Flow Chart

Here, the methodology used within *Chapter 4* is graphically presented to create maps of environmental suitability for the lion.

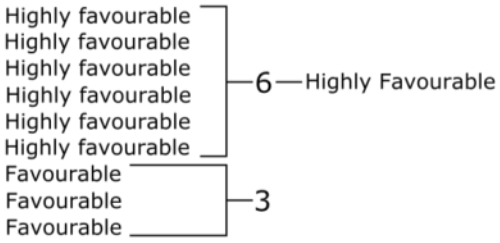

Lion localities
(1135 points)
Present Day
Bioclimatic
Variables
Mid Holocene
Bioclimatic
Variables (9 GCM's)
Last Glacial Maximum
Bioclimatic
Variables (3 GCM's)
MaxEnt
Lion Suitability
Model
Global Environmental
Zone/Strata creation
using methods of
Soteriades et al., (2017)
MaxEnt
Lion Suitability
Threshold
Present Day
GEnZ/GEnS
Mid Holocene
GEnZ/GEnS
(3 GCM's)
Last Glacial Maximum
GEnZ/GEnS
(3 GCM's)
Areas of GEnS/GEnZ
within suitability thresholds
calculated as % area of
modelling extent.
Multiple GCM's reduced to single
favourability output for each
time period
PRESENT CONDITIONS
MID HOLOCENE
LA ST GLACIAL MAXIMUM
Present
Lion Favorability
dictated by GEnS
Mid Holocene
Lion Favorability
dictated by GEnS
Last Glacial Maximum
Lion Favorability
dictated by GEnS
MODEL OUTPUT
Present Day
Lion Distribution
IUCN
Areas of GEnZ within present
day IUCN lion distribution
calculated as % area of
modelling extent
Present Day
GEnZ/GEnS
COMPARATIVE
ANALYSIS
MODIS Landcover
Classes, Present Day
The representation of modis
landcover classes within GEnS/Z
categories within the modelling
extent.

Reducing multiple global climate models to a single map output

By using nine global climate models for the mid-Holocene, and three models for the Last Glacial Maximum, some of the uncertainty portrayed in the climate data and MaxEnt modelling is captured. It is advantageous however, to display a single modelled output of favourable climatic conditions for the lion during these time periods. Lion favourability categories are assigned to global environmental strata for each suitability model, and then apply the following rules to produce the final output.

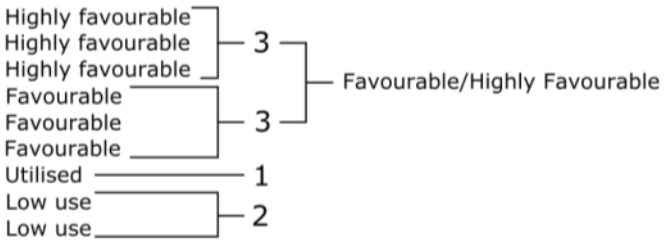
- Lion favourability is assigned to the most commonly found category e.g. if two out of three models are 'unsuitable', this category is assigned (modal winner).

  Example:



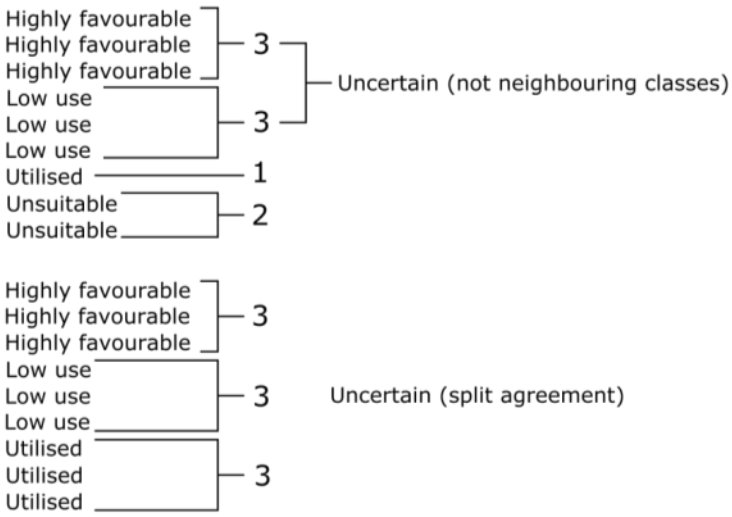
- If there is no modal winner, but there are two neighbouring classes with equal support over other classes, the favourability rating is split. So if four models are highly favourable, four models are favourable, and one model is suitable, then the category will be assigned as 'favourable/highly favourable'.

  Example:



- If there is equal support for two classes that are not neighbouring, support for more than two classes, or no consensus between models, then the category is assigned to uncertain, given the wide discrepancy of results.

  Example:


Highly favourable
Highly favourable
Highly favourable
3
Low use
Low use
Low use
3
Uncertain (not neighbouring classes)
Utilised
1
Unsuitable
Unsuitable
2
Highly favourable
Highly favourable
Highly favourable
3
Low use
Low use
Low use
3
Uncertain (split agreement)
Utilised
Utilised
Utilised
3

Appendix S3: MaxEnt Model Parameters and Results

Final model parameters (using all localities)

- 1133 presence records used for training.
- 100000 points used to determine the Maxent distribution (background points).
- Environmental layers used (continuous):
    - ai_5m_ext
    - pet_sd_5m_ext
    - tm_dab05mext
    - tm_sd_5m_ext
    - Environmental layer extent: -19°E, 94°W, - 36°S, 50°N
- Regularization values: linear/quadratic/product: 0.050, categorical: 0.250, threshold: 1.000, hinge: 0.500
- Feature types used:
    - Hinge
    - Product
    - Linear
    - Threshold
    - Quadratic.
- Response curves: true
- Jackknife: true
- Remove duplicates: false
- Beta multiplier: 2.0
- Maximum background: 100000
- Add samples to background: false

Whilst the model displayed in Fig.1 is raw output, threshold values of bioclimatic suitability were necessarily obtained using logistic output.

Model validation

Models were run to ascertain Area under the Receiver Operator Curve (AUC) using 10 fold cross validation within MaxEnt under the same parameters as the final model. The average test AUC for the replicate runs was 0.923.

In addition, spatially independent cross validation was performed using the ENMeval package (Muscarella et al., 2014) within R (Team, 2015), using the 'checkerboard2' method. Parameters were consistent with those used in the final model. The model AUC score from spatially independent cross-validation was 0.818.

Raw Output and Model Thresholds

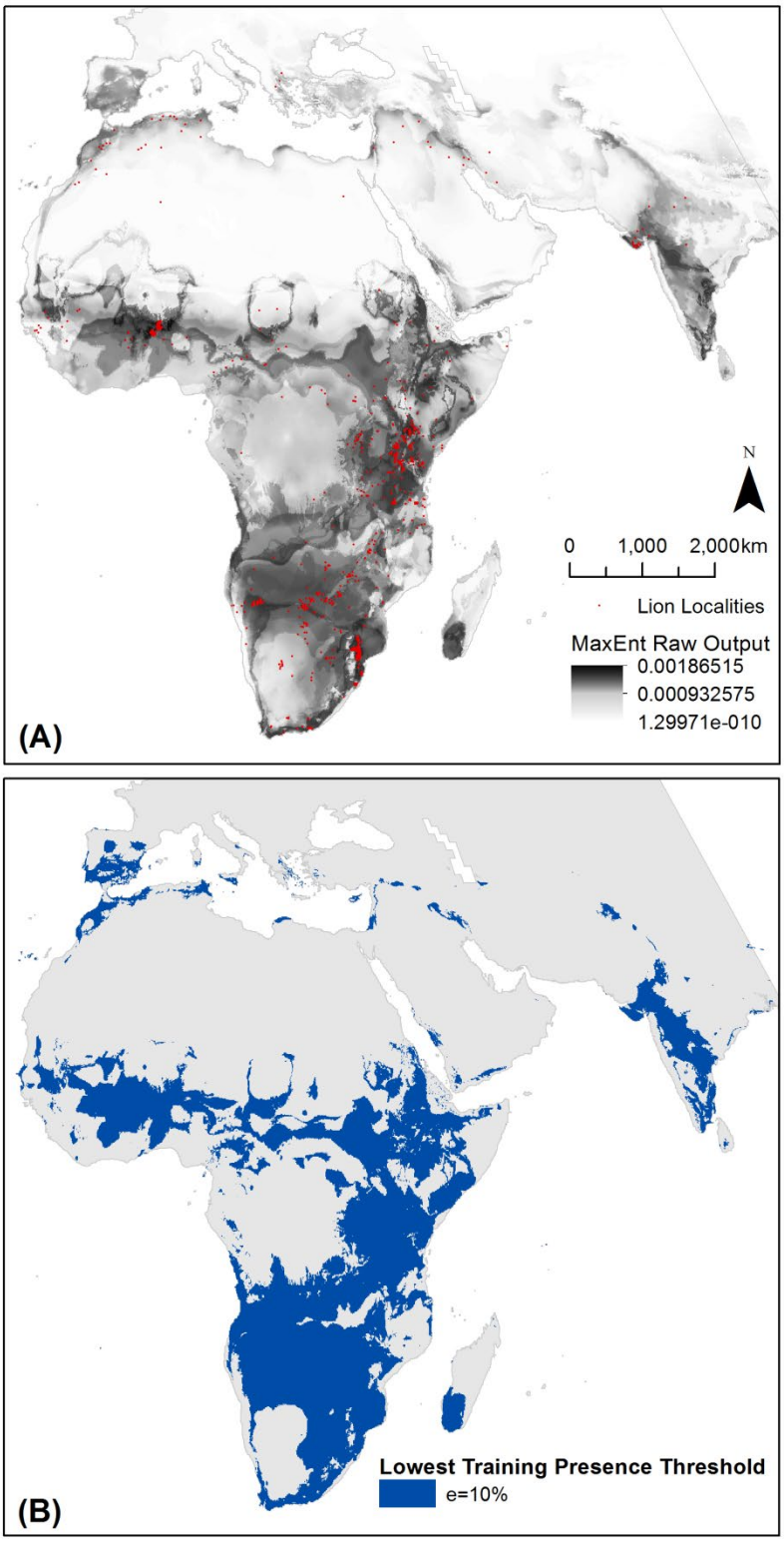
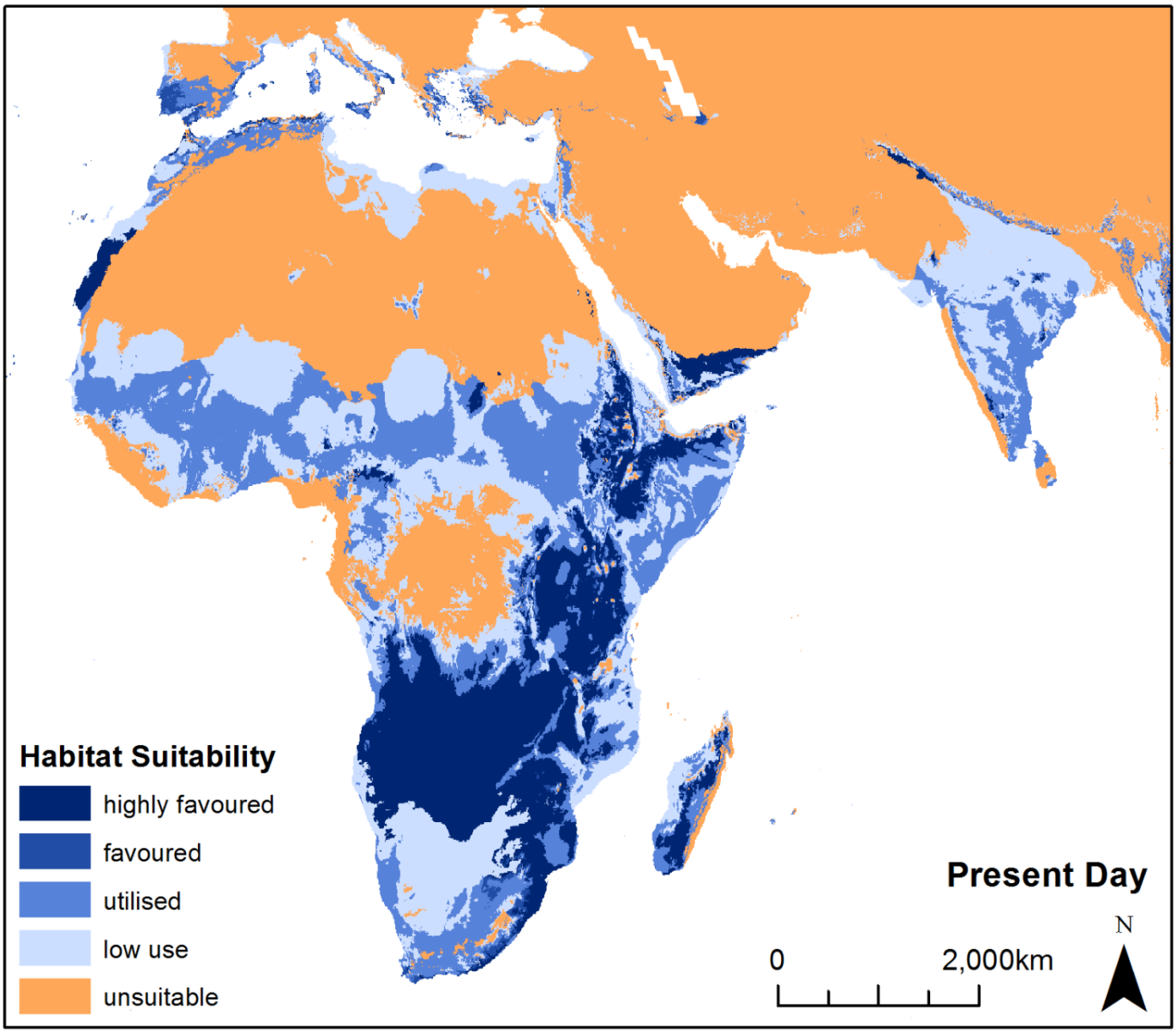
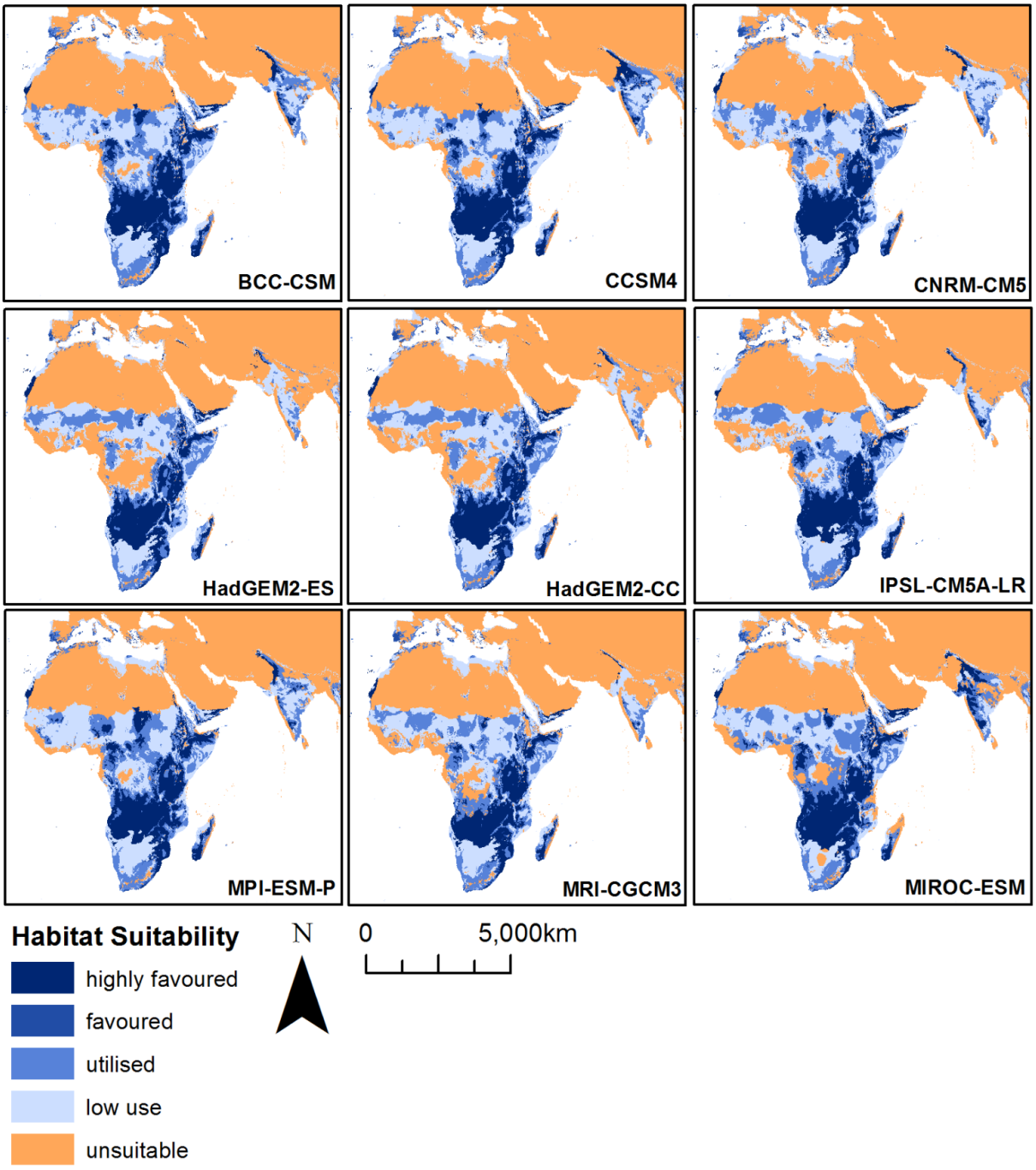


Figure 7: MaxEnt raw output (**A**) shows the present day suitability of Africa and the Near East for the lion based upon current and historic lion localities. MaxEnt suitability is converted into a threshold value (**B**) to enable comparison with Global Environmental Zones and Strata.

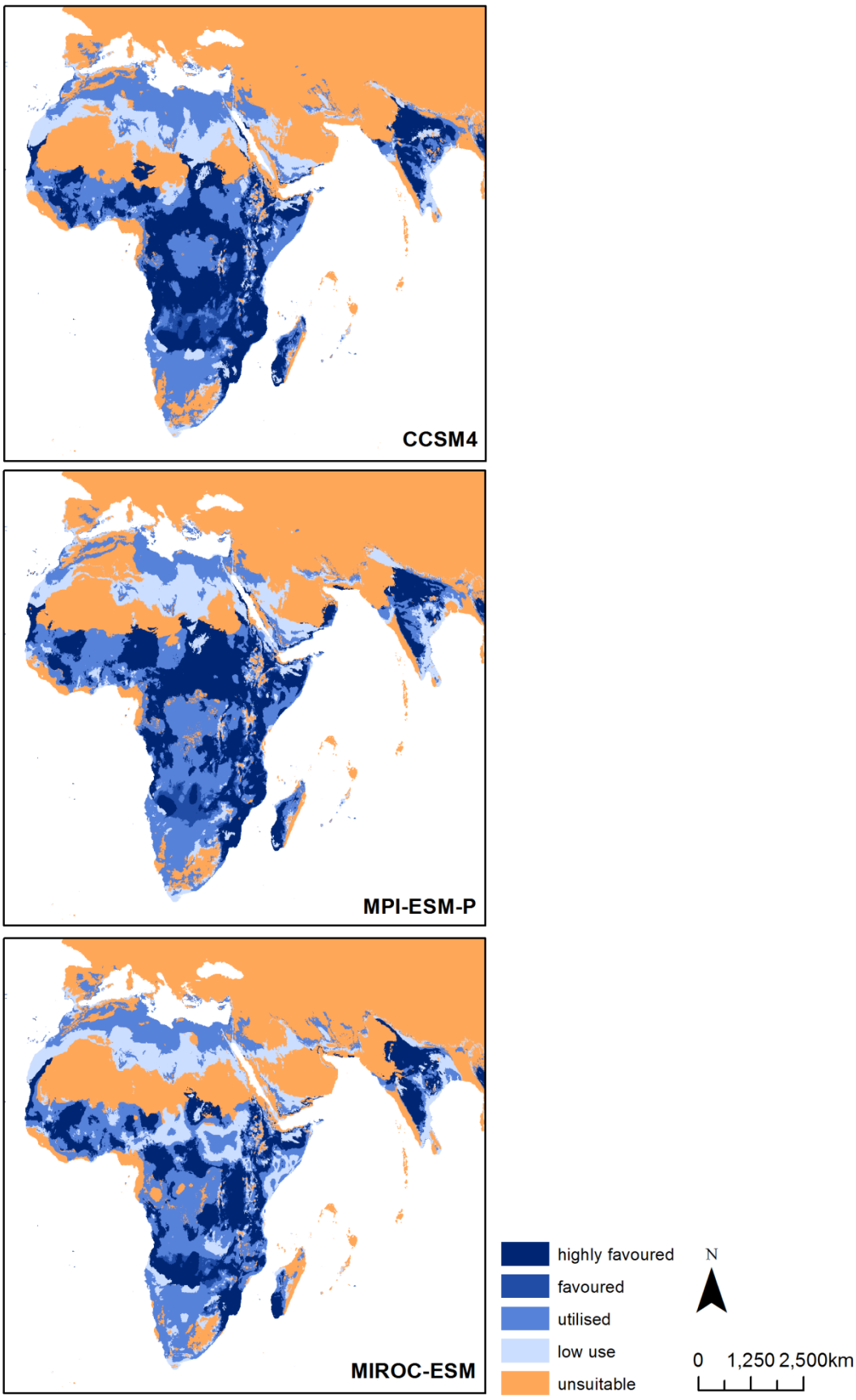
Appendix S4: All Lion Suitability Models for the Present Day, Mid-Holocene and Last Glacial Maximum



Present day lion suitability within the framework of Global Environmental Strata. Suitability is based upon both Equal Training Sensitivity and Specificity (ETSS) and Maximum Training Sensitivity plus Specificity (MTSS) MaxEnt species distribution model suitability thresholds.



Mid-Holocene lion suitability presented for all climate model reconstructions of Global Environmental Strata (GEnS). Lion suitability is based upon both Equal Training Sensitivity and Specificity (ETSS) and Maximum Training Sensitivity plus Specificity (MTSS) MaxEnt species distribution model suitability thresholds.



Last Glacial Maximum lion suitability presented for all global climate model reconstructions of Global Environmental Strata (GEnS). Lion suitability is based upon both Equal Training Sensitivity and Specificity (ETSS) and Maximum Training Sensitivity plus Specificity (MTSS) MaxEnt species distribution model suitability thresholds.

Appendix S5 Saharan Rock Art reference table

Proxy evidence of lions within the Sahara during the African Humid Period ~12ka-6ka from depictions in African Rock Art. Other species present within the rock art, including wild species (bold).

British Museum Reference	Country	Region	Rock Art Site	Other Species Present
2013,2034.11784	Libya	Tassili n Ajjer	Tadrart Acacus, Lion Rock	
2013,2034.4770	Algeria	Tassili n Ajjer	Tadrart Acacus	
2013,2034.4788	Algeria	Tassili n Ajjer	Tadrart Acacus	
2013,2034.4787	Algeria	Tassili n Ajjer	Tadrart Acacus	
2013,2034.2000	Libya	Tassili n Ajjer	Tadrart Acacus, Wadi Tihedene	
2013,2034.1726	Libya	Tassili n Ajjer	Tadrart Acacus, Wadi Inelighi	**Elephant**
2013,2034.481	Libya	Tassili n Ajjer	Tadrart Acacus, Acacus Mountains	**Hippopotamus**
2013,2034.1469	Libya	Tassili n Ajjer	Tadrart Acacus, Lion Rock	Sheep/goat
2013,2034.11783	Libya	Tassili n Ajjer	Tadrart Acacus, Lion Rock	Camel
2013,2034.4759	Algeria	Tassili n Ajjer	Tadrart Acacus	
2013,2034.11778	Libya	Tassili n Ajjer	Tadrart Acacus, Lion Rock	Camel/**Giraffe**
2013,2034.1464	Libya	Tassili n Ajjer	Tadrart Acacus, Lion Rock	**Antelope**
2013,2034.4763	Algeria	Tassili n Ajjer	Tadrart Acacus	Cow/**buffalo**, **ostrich**, **roan antelope**, **giraffe**
2013,2034.4864	Algeria	Tassili n Ajjer	Oued Afar	
2013,2034.5006	Algeria	Tassili n Ajjer	Oued Djerat	
2013,2034.12631	Algeria	Tassili n Ajjer	Oued Djerat	Yes (undefined)
2013,2034.5099	Algeria	Tassili n Ajjer	Oued Djerat	
2013,2034.23850	Algeria	Tassili n Ajjer	Tassili n'Ajer, Afara Plain	Unidentified animal
2013,2034.2095	Libya	Tassili n Ajjer	Tassili n'Ajer, Emi ‘n’ Eher	Cow
2013,2034.4200	Algeria	Tassili n Ajjer	Tassili n'Ajer, Tin Aboteka	
2013,2034.2084	Libya	Tassili n Ajjer	Tassili n'Ajer, Emi ‘n’ Eher	Cow, horse, goat, horse, unidentified quadruped
2013,2034.9166	Niger	Aïr Mountains	Tanakom	
2013,2034.9165	Niger	Aïr Mountains	Tanakom	
2013,2034.9158	Niger	Aïr Mountains	Tanakom	
2013,2034.9164	Niger	Aïr Mountains	Tanakom	**Giraffe**, **warthog**, unidentified quadruped
2013,2034.9288	Niger	Aïr Mountains	Tanakom	**Ostrich**, barbary sheep, unidentified animals
2013,2034.9198	Niger	Aïr Mountains	Tanakom	Cow, **ostrich**/**flamingo**, unidentified quadrupeds
2013,2034.9257	Niger	Aïr Mountains	Tanakom	Cow, **antelope**, **ostrich**
2013,2034.9801	Niger	Aïr Mountains	Iwellene	
2013,2034.9840	Niger	Aïr Mountains	Iwellene	
2013,2034.9839	Niger	Aïr Mountains	Iwellene	**Giraffe**, cow
2013,2034.9862	Niger	Aïr Mountains	Iwellene	**Ostrich**
2013,2034.11033	Niger	Aïr Mountains	Telahlaghe	
2013,2034.11287	Niger	Aïr Mountains	Dabous	
2013,2034.10862	Niger	Aïr Mountains	Dabous	
2013,2034.10861	Niger	Aïr Mountains	Dabous	
2013,2034.10837	Niger	Aïr Mountains	Dabous	**Antelope**, **giraffe**
2013,2034.10859	Niger	Aïr Mountains	Dabous	**Antelope**, **giraffe**, cow
2013,2034.11128	Niger	Aïr Mountains	Western Air	
2013,2034.10183	Niger	Aïr Mountains	Mammanet	Giraffe

2013,2034.9981	Niger	Aïr Mountains	Mammanet	Unidentified quadruped
2013,2034.10186	Niger	Aïr Mountains	Mammanet	**Giraffe**
2013,2034.9431	Niger	Aïr Mountains	Tagueit	**Giraffe**, horse, **rhino**
2013,2034.11125	Niger	Aïr Mountains	Indakate	Horse, dog
2013,2034.11115	Niger	Aïr Mountains	Indakate	Horse, cow, **antelope** dog
2013,2034.116	Egypt	Wadi el-Obeid	Wadi el-Obeid	
2013,2034.111	Egypt	Wadi el-Obeid	Wadi el-Obeid	Horse

Appendix S6: Modis Landcover Classes Table and GEnS/MODIS Comparison Figure

The relationship between each environmental strata and environmental zone is displayed, alongside the characteristic Modis natural land cover of each strata within the modelling extent. The percentage of each strata is included within each modelled lion habitat threshold, and within the IUCN extant distribution of the lion.

GEnS	GEnZ	Modis Natural Cover Characteristics	Lowest Presence Threshold, e=10%	IUCN Extant Distribution
1	A. Arctic	Neglible/No coverage	NA	NA
2	A. Arctic	Neglible/No coverage	NA	NA
3	B. Arctic	Neglible/No coverage	NA	NA
4	B. Arctic	Neglible/No coverage	0.00	0.00
5	B. Arctic	Neglible/No coverage	0.00	0.00
6	C. Extremely cold and wet	Neglible/No coverage	0.00	0.00
7	C. Extremely cold and wet	Neglible/No coverage	0.00	0.00
8	D. Extremely cold and wet	Neglible/No coverage	0.00	0.63
9	D. Extremely cold and wet	Neglible/No coverage	0.00	0.06
10	D. Extremely cold and wet	Neglible/No coverage	0.00	0.00
11	F. Extremely cold and mesic	Neglible/No coverage	0.00	0.00
12	F. Extremely cold and mesic	Neglible/No coverage	0.00	0.00
13	E. Cold and wet	Neglible/No coverage	0.00	0.18
14	E. Cold and wet	Neglible/No coverage	0.00	0.29
15	F. Extremely cold and mesic	Neglible/No coverage	NA	NA
16	F. Extremely cold and mesic	Barren_sparse and Grasslands	0.00	0.00
17	F. Extremely cold and mesic	Neglible/No coverage	0.00	0.00
18	F. Extremely cold and mesic	Neglible/No coverage	0.00	0.00
19	F. Extremely cold and mesic	Grasslands	0.00	0.00
20	F. Extremely cold and mesic	Grasslands and Barren_sparse	0.00	0.00
21	F. Extremely cold and mesic	Neglible/No coverage	NA	NA
22	F. Extremely cold and mesic	Neglible/No coverage	0.00	0.00
23	C. Extremely cold and wet	Neglible/No coverage	NA	NA
24	E. Cold and wet	Neglible/No coverage	0.00	0.00
25	F. Extremely cold and mesic	Neglible/No coverage	NA	NA
26	F. Extremely cold and mesic	Neglible/No coverage	0.33	0.41
27	F. Extremely cold and mesic	Grasslands and Barren_sparse	0.00	0.00
28	F. Extremely cold and mesic	Neglible/No coverage	NA	NA
29	F. Extremely cold and mesic	Grasslands	0.00	0.00
30	G. Cold and mesic	Grasslands	0.00	0.00
31	G. Cold and mesic	Neglible/No coverage	NA	NA
32	E. Cold and wet	Neglible/No coverage	0.00	0.00
33	G. Cold and mesic	Neglible/No coverage	NA	NA
34	G. Cold and mesic	Grasslands	0.00	0.00
35	G. Cold and mesic	Neglible/No coverage	0.00	0.00
36	G. Cold and mesic	Grasslands	0.00	0.00
37	G. Cold and mesic	Grasslands and Barren_sparse	0.00	0.00

38	G. Cold and mesic	Forest covers	0.55	0.18
39	E. Cold and wet	Neglible/No coverage	NA	NA
40	G. Cold and mesic	Grasslands and Forest Covers	0.00	0.00
41	G. Cold and mesic	Grasslands	0.00	0.00
42	G. Cold and mesic	Grasslands and Forest Covers	2.72	0.85
43	H. Cool temperate and dry	Grasslands	0.00	0.00
44	G. Cold and mesic	Forest covers and Grasslands	0.00	0.00
45	H. Cool temperate and dry	Grasslands and Barren_sparse	0.71	0.00
46	H. Cool temperate and dry	Neglible/No coverage	0.00	0.00
47	G. Cold and mesic	Forest Covers	0.43	0.03
48	G. Cold and mesic	Neglible/No coverage	0.00	0.00
49	J. Cool temperate and moist	Forest Covers	4.86	0.86
50	H. Cool temperate and dry	Grasslands, some Barren_sparse	0.00	0.00
51	H. Cool temperate and dry	Forest Covers and Grasslands	0.00	0.00
52	H. Cool temperate and dry	Neglible/No coverage	0.00	0.00
53	J. Cool temperate and moist	Neglible/No coverage	0.00	0.00
54	H. Cool temperate and dry	Grasslands, some Barren_sparse and Open Shrublands	0.33	0.00
55	J. Cool temperate and moist	Forest Covers	0.51	0.12
56	H. Cool temperate and dry	Neglible/No coverage	43.98	0.00
57	H. Cool temperate and dry	Grasslands and Forest Covers	0.00	0.00
58	I. Cool temperate and xeric	Grasslands, some Barren_sparse	0.00	0.00
59	I. Cool temperate and xeric	Grasslands, some Open_shrublands	0.00	0.00
60	J. Cool temperate and moist	Forest Covers	2.55	0.44
61	J. Cool temperate and moist	Forest Covers and Grasslands	2.02	0.05
62	J. Cool temperate and moist	Neglible/No coverage	0.00	0.00
63	I. Cool temperate and xeric	Grasslands and Barren_sparse	0.00	0.00
64	I. Cool temperate and xeric	Grasslands, some Open_shrublands	0.00	0.00
65	I. Cool temperate and xeric	Grasslands and Open_shrublands, some Barren_sparse	9.49	0.00
66	K. Warm temperate and mesic	Forest Covers, Grasslands and Woody_savannas	6.02	0.29
67	K. Warm temperate and mesic	Forest Covers	10.26	0.92
68	K. Warm temperate and mesic	Neglible/No coverage	0.00	0.00
69	I. Cool temperate and xeric	Barren_sparse, some Grasslands and Open_shrublands	0.00	0.00
70	K. Warm temperate and mesic	Open Shrublands and Grasslands, some Barren_sparse	8.02	0.00
71	K. Warm temperate and mesic	Woody_savannas, Grasslands, Forest Covers, Open_shrublands	23.42	0.34
72	K. Warm temperate and mesic	Neglible/No coverage	0.00	0.00
73	K. Warm temperate and mesic	Forest Covers	12.16	1.64
74	K. Warm temperate and mesic	Barren_sparse, Open_shrublands and Grasslands	0.00	0.00
75	K. Warm temperate and mesic	Open Shrublands, some Barren_sparse	44.48	0.02
76	K. Warm temperate and mesic	Woody_savannas and Grasslands	50.46	1.42
77	K. Warm temperate and	Neglible/No coverage	0.00	0.00

	mesic			
78	L. Warm temperate and xeric	Open_shrublands, some Barren_sparse	46.14	0.05
79	L. Warm temperate and xeric	Barren_sparse and Open_shrublands	0.21	0.00
80	K. Warm temperate and mesic	Forest Covers	6.41	0.14
81	K. Warm temperate and mesic	Forest Covers, Savannas and Woody_savannas	42.63	10.08
82	L. Warm temperate and xeric	Woody_savannahs, Grasslands and Savannas	66.04	1.53
83	N. Hot and dry	Neglible/No coverage	0.00	0.00
84	L. Warm temperate and xeric	Open_shrublands, some Barren_sparse and Grasslands	48.22	1.70
85	N. Hot and dry	Forest Covers	1.04	0.00
86	L. Warm temperate and xeric	Barren_sparse and Open_shrublands	5.39	0.00
87	N. Hot and dry	Forest Covers, Savannas and Woody_savannas	50.15	10.66
88	N. Hot and dry	Savannas and Woody_savannas, some Grasslands	88.37	9.20
89	L. Warm temperate and xeric	Open_shrublands and Barren_sparse	36.49	2.36
90	N. Hot and dry	Neglible/No coverage	13.82	0.00
91	N. Hot and dry	Open_shrublands and Barren_sparse, some Savannas	26.38	4.39
92	N. Hot and dry	Barren_sparse, some Open_shrublands	1.91	0.00
93	N. Hot and dry	Forest Covers, some Woody_savannas	7.98	1.61
94	N. Hot and dry	Woody_savannas and Savannas, some Grasslands	81.18	21.41
95	M. Hot and mesic	Forest Covers	8.16	0.00
96	N. Hot and dry	Barren_sparse, Open_shrublands and Grasslands	26.06	13.72
97	N. Hot and dry	Woody_savannas and Savannas	87.20	28.22
98	M. Hot and mesic	Forest Covers and Woody_savannas	46.60	1.37
99	O. Hot and arid	Barren_sparse, some Open_shrublands	0.76	0.00
100	M. Hot and mesic	Savannas, some Barren_sparse, Open_shrublands, Grasslands and Woody_savannas	82.84	37.44
101	M. Hot and mesic	Woody_savannas, some Savannas and Forest Covers	80.55	12.87
102	M. Hot and mesic	Forest Covers, some Woody_savannas	1.47	0.00
103	O. Hot and arid	Barren_sparse, some Open_shrublands	1.98	0.00
104	M. Hot and mesic	Savannas, Woody_savannas, Barren_sparse, Grasslands and Open_shrublands	80.56	32.37
105	M. Hot and mesic	Woody_savannas and Forest Covers, some Savannas	41.85	11.14
106	M. Hot and mesic	Forest Covers, some Woody_savannas	5.41	0.00
107	O. Hot and arid	Barren_sparse, some Open_shrublands	0.55	0.00
108	R. Extremely hot and moist	Woody_savannas and Forest Covers, some Savannas	12.72	18.28
109	R. Extremely hot and moist	Savannas, Woody_savannas, Open_shrublands, Grasslands and Barren_sparse	59.71	33.97
110	P. Extremely hot and arid	Barren_sparse, some Open_shrublands	0.79	0.00
111	R. Extremely hot and moist	Forest Covers, some Woody_savannas	3.92	0.00
112	R. Extremely hot and moist	Forest Covers, some Woody_savannas	3.41	6.99

113	R. Extremely hot and moist	Woody_savannas and Savannas	28.53	19.06
114	R. Extremely hot and moist	Forest Covers, some Woody_savannas	0.98	0.00
115	R. Extremely hot and moist	Forest Covers and Woody_savannas	1.38	0.00
116	P. Extremely hot and arid	Barren_sparse, some Open_shrublands	1.69	0.00
117	R. Extremely hot and moist	Forest Covers, some Woody_savannas	1.55	0.00
118	R. Extremely hot and moist	Savannas and Woody_savannas, some Forest Covers	26.45	15.10
119	Q. Extremely hot and xeric	Grasslands, Open_shrublands and Savannas, some Woody_savannas	38.47	15.94
120	R. Extremely hot and moist	Forest Covers and Woody_savannas	0.00	0.00
121	Q. Extremely hot and xeric	Barren_sparse, some Open_shrublands and Grasslands	0.12	0.02
122	Q. Extremely hot and xeric	Savannas and Woody_savannas	47.04	13.84
123	Q. Extremely hot and xeric	Savannas, Grasslands and Open_shrublands, some Barren_sparse	53.49	17.37
124	Q. Extremely hot and xeric	Barren_sparse	3.65	0.01
125	Q. Extremely hot and xeric	Barren_sparse and Grasslands, some Open_shrublands	24.21	1.68

Highly Favoured	>80
Favoured	>60
Utilised	>40
Low Use	>10
Unsuitable	<10

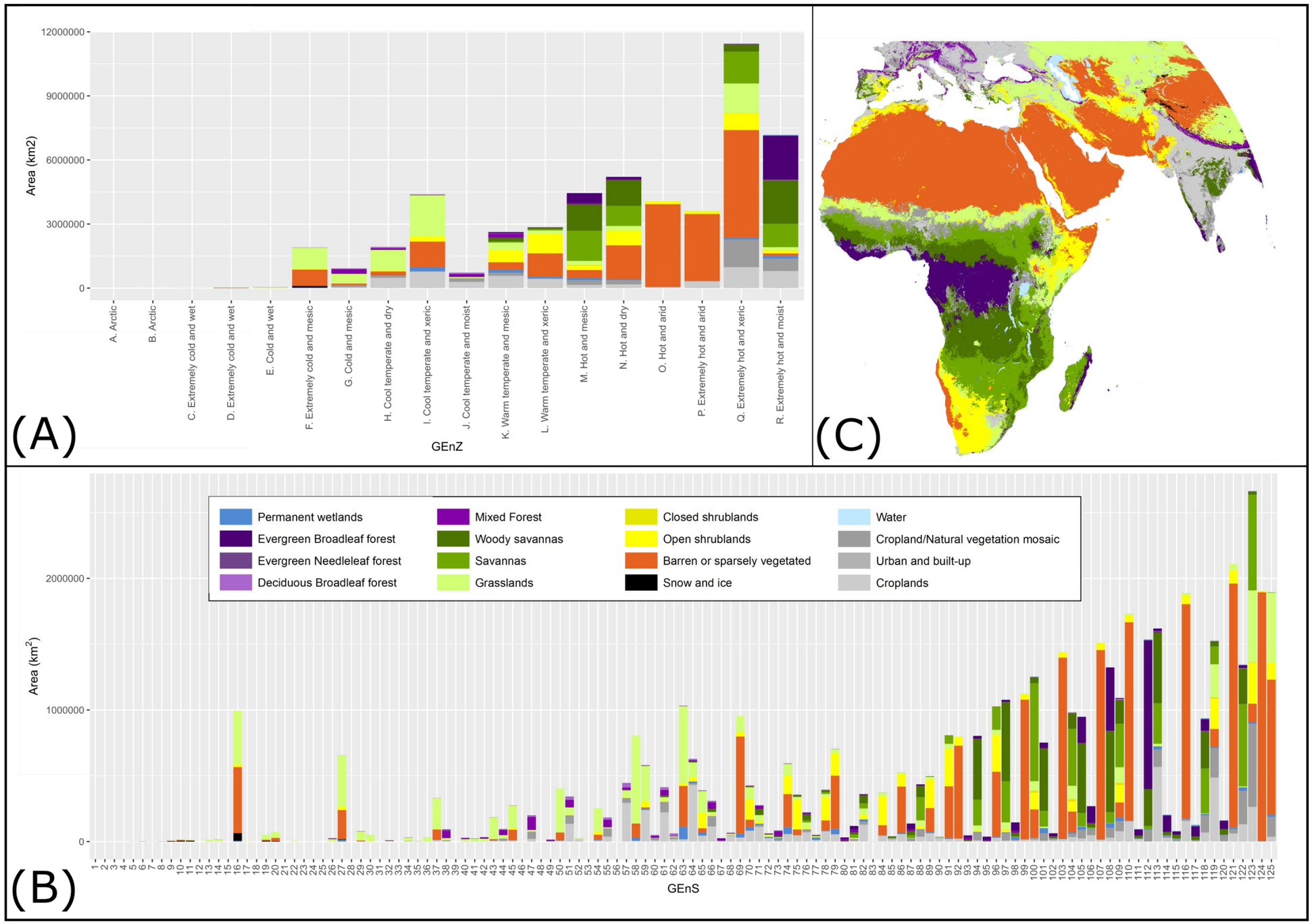


Figure S1: The proportion of both global environmental zones (A) and environmental strata (B) within the study extent (C) is displayed for the present day. Colours represent the MODIS landcover classes which characterise the zones and strata within the study extent. By comparing MODIS landcover to environmental zones and strata for the present day, we gain insight into the probable landcovers these bioclimatic categories represent for the mid-Holocene and Last Glacial Maximum. Croplands, Urban and built-up and Cropland/Natural vegetation mosaics are represented by greys, and are not useful in determining past land covers.

References

Antunes A., Troyer J.L., Roelke M.E., Pecon-Slattery J., Packer C., Winterbach C., Winterbach H., Hemson G., Frank L., Stander P., Siefert L., Driciru M., Funston P.J., Alexander K.A., Prager K.C., Mills G., Wildt D., Bush M., O'Brien S.J., & Johnson W.E. (2008) The evolutionary dynamics of the lion Panthera leo revealed by host and viral population genomics. *PLoS Genetics*, **4**, 1–11.

Banerjee K. & Jhala Y. V. (2012) Demographic parameters of endangered Asiatic lions (Panthera leo persica) in Gir Forests, India. *Journal of Mammalogy*, **93**, 1420–1430.

Barker P. & Gasse F. (2003) New evidence for a reduced water balance in East Africa during the Last Glacial Maximum: Implication for model-data comparison. *Quaternary Science Reviews*, **22**, 823–837.

Barnett R., Shapiro B., Barnes I., Ho S.Y.W., Burger J., Yamaguchi N., Higham T.F.G., Wheeler H.T., Rosendahl W., Sher A. V, Sotnikova M., Kuznetsova T., Baryshnikov G.F., Martin L.D., Harington C.R., Burns J.A., & Cooper A. (2009) Phylogeography of lions (Panthera leo ssp.) reveals three distinct taxa and a late Pleistocene reduction in genetic diversity. *Molecular ecology*, **18**, 1668–77.

Barnett R., Yamaguchi N., Shapiro B., Ho S.Y.W., Barnes I., Sabin R., Werdelin L., Cuisin J., & Larson G. (2014) Revealing the maternal demographic history of Panthera leo using ancient DNA and a spatially explicit genealogical analysis. *BMC Evolutionary Biology*, **14**, 70.

Bartosiewicz L. (2009) A lion's share of attention: archaeozoology and the historical record. *Acta Archaeologica*, **60**, 275–289.

Bauer H., de Iongh H.H., & Di Silvestre I. (2003) Lion (Panthera leo) social behaviour in the West and Central African savannah belt. *Mammalian Biology*, **68**, 239–243.

Bauer H., Packer C., Funston P.F., Henschel P., & Nowell K. (2016) Available at: http://www.iucnredlist.org/details/summary/15951/0.

Bertola L.D., Jongbloed H., van der Gaag K.J., de Knijff P., Yamaguchi N., Hooghiemstra H., Bauer H., Henschel P., White P.A., Driscoll C.A., Tende T., Ottosson U., Saidu Y., Vrieling K., & de Iongh H.H. (2016) Phylogeographic Patterns in Africa and High Resolution Delineation of Genetic Clades in the Lion (Panthera leo). *Scientific Reports*, **6**, 1–11.

Black S.A., Fellous A., Yamaguchi N., & Roberts D.L. (2013) Examining the Extinction of the Barbary Lion and Its Implications for Felid Conservation. *PLoS ONE*, **8**, 1–12.

Celesia G.G., Townsend Peterson a., Kerbis Peterhans J.C., & Gnoske T.P. (2010) Climate and landscape correlates of African lion (Panthera leo) demography. *African Journal of Ecology*, **48**, 58–71.

Channan S., Collins K., & Emanuel W.R. (2014) Global mosaics of the standard MODIS land cover type data. .

Chevalier M., Brewer S., & Chase B.M. (2017) Qualitative assessment of PMIP3 rainfall simulations across the eastern African monsoon domains during the mid-Holocene and the Last Glacial Maximum. *Quaternary Science Reviews*, **156**, 107–120.

Constable H., Guralnick R., Wieczorek J., Spencer C., & Peterson A.T. (2010) VertNet: A new model for biodiversity data sharing. *PLoS Biology*, **8**, 1–4.

Cooper D.M., Dugmore A.J., Gittings B.M., Scharf A.K., Wilting A., & Kitchener A.C. (2016) Predicted Pleistocene-Holocene range shifts of the tiger (Panthera tigris). *Diversity and Distributions*, **22**, 1199–1211.

Cooper D.M., Dugmore A.J., Kitchener A.C., Metzger M.J., & Trabucco A. (2019) Global Environmental Stratification for the mid-Holocene and Last Glacial Maximum, based upon PMIP3 experiments. .

Costa G.C., Nogueira C., Machado R.B., & Colli G.R. (2010) Sampling bias and the use of ecological niche modeling in conservation planning: A field evaluation in a biodiversity hotspot. *Biodiversity and Conservation*, **19**, 883–899.

Cozzi G., Broekhuis F., Mcnutt J.W., & Schmid B. (2013) Comparison of the effects of artificial and

natural barriers on large African carnivores: Implications for interspecific relationships and connectivity. *Journal of Animal Ecology*, **82**, 707–715.

Cross P.C., Heisey D.M., Bowers J.A., Hay C.T., Wolhuter J., Buss P., Hofmeyr M., Michel A.L., Bengis R.G., Bird T.L.F., Du Toit J.T., & Getz W.M. (2009) Disease, predation and demography: Assessing the impacts of bovine tuberculosis on African buffalo by monitoring at individual and population levels. *Journal of Applied Ecology*, **46**, 467–475.

Drake N.A., Blench R.M., Armitage S.J., Bristow C.S., & White K.H. (2011) Ancient watercourses and biogeography of the Sahara explain the peopling of the desert. *Proceedings of the National Academy of Sciences*, **108**, 458–462.

Dunne J., Evershed R.P., Salque M., Cramp L., Bruni S., Ryan K., Biagetti S., & di Lernia S. (2012) First dairying in green Saharan Africa in the fifth millennium BC. *Nature*, **486**, 390–394.

Faith J.T. (2014) Late Pleistocene and Holocene mammal extinctions on continental Africa. *Earth-Science Reviews*, **128**, 105–121.

Fei S. & Yu F. (2016) Quality of presence data determines species distribution model performance: a novel index to evaluate data quality. *Landscape Ecology*, **31**, 31–42.

Frank E., Hall M.A., & Witten I.H. (2016) *The WEKA Workbench. Online Appendix for "Data Mining: Practical Machine Learning Tools and Techniques."*

Friedl M.A., Sulla-Menashe D., Tan B., Schneider A., Ramankutty N., Sibley A., & Huang X. (2010) MODIS Collection 5 global land cover: Algorithm refinements and characterization of new datasets. *Remote Sensing of Environment*, **114**, 168–182.

Funston P.J., Mills M.G.L., Richardson P.R.K., & van Jaarsveld A.S. (2003) Reduced dispersal and opportunistic territory acquisition in male lions (Panthera leo). *Journal of Zoology*, **259**, 131–142.

Galvin E. (2018) Available at: https://africanrockart.britishmuseum.org/.

Geffen E., Anderson M.J., & Wayne R.K. (2004) Climate and habitat barriers to dispersal in the highly mobile grey wolf. *Molecular ecology*, **13**, 2481–90.

Hickie J.L.D. (2016) Environmental stratification to model shifts in the historic distribution of the giant panda (Ailuropoda melanoleuca) (Masters dissertation). *https://www.era.lib.ed.ac.uk/handle/1842/19492*, .

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., & Jarvis A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hoelzmann P., Jolly D., Harrison S.P., Laarif F., Bonnefille R., & Pachur H.. (1998) Mid-Holocene land-surface conditions in northern Africa and the Arabian peninsula: A data set of the analysis of biogeographical feedbacks in the climate system. *Global Biogeochemical Cycles*, **12**, 35–51.

iNaturalist (2015) Available at: iNaturalist.org.

Jennings R.P., Singarayer J., Stone E.J., Krebs-Kanzow U., Khon V., Nisancioglu K.H., Pfeiffer M., Zhang X., Parker A., Parton A., Groucutt H.S., White T.S., Drake N.A., & Petraglia M.D. (2015) The greening of Arabia: Multiple opportunities for human occupation of the Arabian Peninsula during the Late Pleistocene inferred from an ensemble of climate model simulations. *Quaternary International*, **382**, 181–199.

Jones M., Djamali M., Stevens L., Heyvaert V., Askari H., Noorollahi D., & Weeks L. (2013) Mid-Holocene environmental and climatic change in Iran. *Ancient Iran and its Neighbours: Local Developments and Long-range Interactions in the 4th Millenium BC* (ed. by C.A. Petrie), Oxbow books, Oxford.

Kohli B.A., Fedorov V.B., Waltari E., & Cook J.A. (2014) Phylogeography of a Holarctic rodent (Myodes rutilus): testing high-latitude biogeographical hypotheses and the dynamics of range shifts. *Journal of Biogeography*, n/a-n/a.

Kopp G.H., Roos C., Butynski T.M., Wildman D.E., Alagaili A.N., Groeneveld L.F., & Zinner D. (2014) Out of Africa, but how and when? The case of hamadryas baboons (Papio hamadryas). *Journal of Human Evolution*, **76**, 154–164.

Kröpelin S., Verschuren D., Lezine A.-M., Eggermont H., Cocquyt C., Francus P., Cazet J.-P., Fagot M., Rumes B., Russell J.M., Darius F., Conley D.J., Schuster M., von Suchodoletz H., & Engstrom D.R. (2008) Climate-Driven Ecosystem Succession in the Sahara: The Past 6000 Years. *Science*, **320**, 765–768.

Kuper R. & Kröpelin S. (2006) Climate-Controlled Holocene Occupation in the Sahara: Motor of Africa's Evolution. *Science*, **313**, 803–807.

Larrasoaña J.C., Roberts A.P., & Rohling E.J. (2013) Dynamics of Green Sahara Periods and Their Role in Hominin Evolution. *PLoS ONE*, **8**, .

Li J., McCarthy T.M., Wang H., Weckworth B. V., Schaller G.B., Mishra C., Lu Z., & Beissinger S.R. (2016) Climate refugia of snow leopards in High Asia. *Biological Conservation*, **203**, 188–196.

Lorenzen E.D., Heller R., & Siegismund H.R. (2012) Comparative phylogeography of African savannah ungulates. *Molecular Ecology*, **21**, 3656–3670.

Loveridge A.J. & Canney S. (2009) Report to Born Free Foundation on the Lion Distribution and Conservation Modelling Project. .

Martínez-Meyer E., Peterson A.T., & Hargrove W.W. (2004) Ecological niches as stable distributional constraints on mammal species , with implications for Pleistocene extinctions and climate change projections for biodiversity. *Global Ecology and Biogeography*, **13**, 305–314.

Meena V. (2009) Variation in social organisation of lions with particular reference to the Asiatic Lions Panthera leo persica (Carnivora: Felidae) of the Gir forest, India. *Journal of Threatened Taxa*, **1**, 158–165.

Metzger M.J., Brus D.J., Bunce R.G.H., Carey P.D., Gonçalves J., Honrado J.P., Jongman R.H.G., Trabucco A., & Zomer R. (2013a) Environmental stratifications as the basis for national, European and global ecological monitoring. *Ecological Indicators*, **33**, 26–35.

Metzger M.J., Bunce R.G.H., Jongman R.H.G., Sayre R., Trabucco A., & Zomer R. (2013b) A high-resolution bioclimate map of the world: A unifying framework for global biodiversity research and monitoring. *Global Ecology and Biogeography*, **22**, 630–638.

Migliore J., Baumel A., Juin M., Fady B., Roig A., Duong N., & Médail F. (2013) Surviving in Mountain Climate Refugia: New Insights from the Genetic Diversity and Structure of the Relict Shrub Myrtus nivellei (Myrtaceae) in the Sahara Desert. *PLoS ONE*, **8**, 1–10.

Muscarella R., Galante P.J., Soley-Guardia M., Boria R.A., Kass J.M., Uriarte M., & Anderson R.P. (2014) ENMeval: An R package for conducting spatially independent evaluations and estimating optimal model complexity for Maxent ecological niche models. *Methods in Ecology and Evolution*, **5**, 1198–1205.

Peterson A.T., Papeş M., & Soberón J. (2008) Rethinking receiver operating characteristic analysis applications in ecological niche modeling. *Ecological Modelling*, **213**, 63–72.

Phillips S.J. & Dudík M. (2008) Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, **31**, 161–175.

Raxworthy C.J., Ingram C.M., Rabibisoa N., & Pearson R.G. (2007) Applications of ecological niche modeling for species delimitation: a review and empirical evaluation using day geckos (Phelsuma) from Madagascar. *Systematic biology*, **56**, 907–23.

Ray J.C., Hunter L.T.B., & Zigouris J. (2005) Setting Conservation and Research Priorities for Larger African Carnivores. p.203. Available at: http://www.catsg.org/cheetah/05_. *Wildlife Conservation Society*, **24**, 1–203.

Rebelo H., Froufe E., Brito J.C., Russo D., Cistrone L., Ferrand N., & Jones G. (2012) Postglacial colonization of Europe by the barbastelle bat: agreement between molecular data and past predictive modelling. *Molecular Ecology*, **21**, 2761–74.

Scheffer M., Hirota M., Holmgren M., Van Nes E.H., & Chapin F.S. (2012) Thresholds for boreal biome transitions. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 21384–9.

Schnitzler A.E. (2011) Past and present distribution of the North African-Asian lion subgroup: a review. *Mammal Review*, **41**, 220–243.

Singarayer J.S. & Burrough S.L. (2015) Interhemispheric dynamics of the African rainbelt during the late Quaternary. *Quaternary Science Reviews*, **124**, 48–67.

Soteriades A.D., Murray-Rust D., Trabucco A., & Metzger M.J. (2017) Understanding global climate change scenarios through bioclimate stratification. *Environmental Research Letters*, **12**, 1–10.

Team R.C. (2015) R: A language and environment for statistical computing. .

Tierney J.E., Pausata F.S.R., & DeMenocal P.B. (2017) Rainfall regimes of the Green Sahara. *Science Advances*, **3**, e1601503.

Timmermann A. & Friedrich T. (2016) Late Pleistocene climate drivers of early human migration.

Nature, **538**, 92–95.
Townsend Peterson A., Radocy T., Hall E., Kerbis Peterhans J.C., & Celesia G.G. (2014) The potential distribution of the Vulnerable African lion Panthera leo in the face of changing global climate. *Oryx*, **48**, 1–10.
Varela S., Lobo J.M., Rodríguez J., & Batra P. (2010) Were the Late Pleistocene climatic changes responsible for the disappearance of the European spotted hyena populations? Hindcasting a species geographic distribution across time. *Quaternary Science Reviews*, **29**, 2027–2035.
Willis K.J., Bennett K.D., Burrough S.L., Macias-Fauria M., & Tovar C. (2013) Determining the response of African biota to climate change : using the past to model the future. *Philosophical Transactions of the Royal Society B*, 1–9.
Yamaguchi N., Cooper A., Werdelin L., & Macdonald D.W. (2004) Evolution of the mane and group-living in the lion (Panthera leo): A review. *Journal of Zoology*, **263**, 329–342.
Yeakel J.D., Pires M.M., Rudolf L., Dominy N.J., Koch P.L., Guimarães P.R., & Gross T. (2014) Collapse of an ecological network in Ancient Egypt. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 14472–14477.
Zomer R.J., Bossio D.A., Trabucco A., Yuanjie L., Gupta D.C., & Singh V.P. (2007) Trees and Water: Smallholder agroforestry on irrigated lands in Northern India. Columbo, Sri Lanka: International Water Management Institute. .
Zomer R.J., Trabucco A., Bossio D.A., & Verchot L. V. (2008) Climate change mitigation: A spatial analysis of global land suitability for clean development mechanism afforestation and reforestation. *Agriculture, Ecosystems and Environment*, **126**, 67–80.

Chapter 5: Understanding the impact of life history on morphological characteristics.

Preface

This chapter is the first of two which analyse skull morphology data. The extent to which skull morphology of lions and tigers is determined by diet and environment is assessed, to provide crucial context to the subsequent analysis of wild specimens in *Chapter 6*.

Introduction

The aim of this chapter is to examine the effect of captivity on skull morphology of the lion (*Panthera leo*) and tiger (*Panthera tigris*), in order to determine the influence of differing environmental conditions and life histories on skull size and shape. Bone is a phenotypically plastic tissue which is capable of large changes in size and shape in response to a multitude of influences (Currey, 2003). Genes do not directly encode bone shape beyond the patterning of the embryo (Mariani & Martin, 2003). An understanding of the plasticity of skull morphology due to the very different environmental conditions and diet of captivity and the wild allows us to determine the extent to which life history, as opposed to evolutionary history, influences skull morphology in wild populations. This is important because putative subspecies across the geographical ranges of both lions and tigers are identified through morphological studies, which assume a dominant role of evolutionary history in determining differences in skull size and shape. By identifying how life history influences skull measurements, the extent to which an individual's environment may affect skull morphology in wild populations can be assessed.

Use of morphology to differentiate subspecies in big cats – macro/micro evolutionary influences.

Whilst genetic studies have superseded morphological studies as the customary determinant of phylogeny, there still is real value in understanding the relationship between morphology, life history and evolutionary history given the historical importance of morphology in determining putative subspecies and their continuing influence on the policies and practices of tiger conservation. Boundaries of subspecies recognised and defined by morphological differences influence conservation management, and so have a real, practical importance for the future of these charismatic species. Skull morphology has been used to both differentiate (see Mazák & Groves, 2006; Christiansen & Harris, 2009; Mazák, 2010; Yamaguchi *et al*., 2013) or show similarities (see Kitchener, 1999; Mazák, 2010; Yamaguchi *et al*., 2013; Wilting *et al*., 2015) between populations of big cats using statistical techniques applied to skull metrics. Full skeletal growth in lions occurs around 3-4 years of age (Smuts et al., 1978) and life stresses before this age, and in adults, may impact skull development. Diet is considered as one of the greatest influences on the skull morphology of vertebrates (Smuts et al., 1978), and a diet rich in bone and connective tissue creates greater stresses on the skull during mastication and therefore may lead to greater bone strength and muscle mass.

Effects of captivity which highlight the plasticity of skull morphology

Differences between captive and wild lion morphology were described for both pelage and skull dimensions during the early 20th Century (Hollister, 1917). Captive lions and tigers are shown to be more robust, with greater rostral and mastoid breadths, broader zygomatic arch width, and mandibles, and shorter overall skull length (Hollister, 1917; Zuccarelli, 2004; Hartstone-Rose et al., 2014). The mechanical influences of diet on biting and chewing have been hypothesised as the driving cause of morphological differences between wild and captive carnivores (Hollister, 1917; Howell, 1917; O'Regan & Kitchener, 2005). Both lions and tigers in captivity have been shown to have greater calculus build-up on the posterior teeth and higher prevalence of periodontal disease compared to wild specimens due to the lack of abrasion caused by soft diets (Kapoor et al., 2016). Wild specimens have been shown to have greater jaw height and mastoid breadth (Zuccarelli, 2004). Wild specimens of lion have been shown to have greater jaw height and mastoid breadth (Zuccarelli, 2004). They have been shown to possess greater skull dimensions in areas of stress, compared to captive lions which are not subjected to such stresses as a result of softer foods, leading to significant morphological differentiation (Zuccarelli, 2004). Captive lion skulls, whilst more massive for

a given length, weigh less than their wild counterparts due to the softer, more spongy nature of the bone (Howell, 1917). Captivity in American zoos (with a different diet from European zoos and the wild) has been shown to have a greater influence on craniometric shape of lions and tigers than sex (Hartstone-Rose et al., 2014). This is most likely because captive diets for lions and tigers that lack carcass feeding, a common occurrence in North American zoos, are structurally unnatural, even if nutritionally complete, as they are free from bone, skin, connective tissues and organs (Hartstone-Rose et al., 2014). Beyond the mechanical properties of diet, it is possible that captive diets vary from the wild by their nutritional properties. Protein and fat digestibility can vary by the type of processing applied to processed food (Kerr et al., 2013), and between dietary items (Bennett et al., 2010).

This chapter is based on significantly bigger datasets than previous craniometric studies comparing captive and wild specimens, and includes measurements from both wild and captive lion and tiger specimens from all putative subspecies. By comparing the fundamentally different environmental conditions between captive and wild lifestyles, this chapter investigates the relative importance of life history, compared to evolutionary history in determining skull morphology. An understanding of traits with established functional significance would aid the study of patterns of adaptive evolution under climatic constraints in the wild (Thuiller *et al*., 2004).

This new evaluation is structured around four research questions that utilise the profound differences between the life histories of wild and captive tigers and lions, and includes an explicit consideration of sex:

Is there a difference between skulls of captive and wild male lions?
Is there a difference between the skulls of captive and wild female lions?
Is there a difference between the skulls of captive and wild male tigers?
Is there a difference between the skulls of captive and wild female tigers?

Rigorously quantified morphometric tests will establish if there are significant differences, what they are, and thus enable us to explore their likely causes. Crucially this approach will define the empirical basis of any differences, and establish the comparative influence of the environment as opposed to evolutionary history.

Approach and Methods

Data

This chapter examines linear cranial measurements of both lions (500 specimens) and tigers (393 specimens) from museum collections across Europe. Sub-adults have been removed from further processing and analysis as skull development in these individuals was still occurring at the time of death (Smuts et al., 1978). Sub-adults are defined by the cemento-enamel junctions of all canines being visible above the alveoli of a cleaned skull, whilst the basioccipital-basisphenoid suture, and/or frontal suture are still open (Barnett et al., 2008).

74 linear measurements, and cranial volume, are recorded for each specimen, provided by Nobuyuki Yamaguchi, and supplemented by myself, following Barnett et al., (2008) (*see Table S1 of the Supplementary Information*). As a quality control measure I selected five skulls for three repeat measurements on different days, to test for intra-observer measurement error. Measurements were removed from further analysis where mean coefficient of variation was above 1%.

Missing data and imputation

The dataset contains missing values for variables due to specimen incompleteness (missing teeth or mandibles) and damage (accidental or from intrusive methods of previous investigation), yet complete data is required for subsequent ordination techniques. Within the data set used here, 9% of missing data affects 38% of specimens. As a result I use multiple imputation by chained equations (MICE) to account for missing data within the dataset (van Buuren, 2012) which works under the Missing At Random (MAR) assumption (Schafer & Graham, 2002; Azur et al., 2012; Baur et al., 2014; Clavel et al., 2014). The large number of predictors used in the analysis undertaken here make the MAR assumption more plausible (Schafer, 1997; White et al., 2011). However, it is still likely that certain skull elements are Missing Not At Random (MNAR). e.g. multiple measurements missing in the absence of a tooth, MICE has been shown to work as well as or better than other single and multiple imputation techniques, and be robust to violations of the MAR assumption (Clavel et al.,

2014). To counteract gross violations of the MAR assumption, I have removed specimens with missing mandibles from the analyses due to the very high number of related measurements on this skeletal element.

In preparation for imputation, specimens with more than 20% of missing data are removed to improve the plausibility and repeatability of the imputation procedure. I include all remaining available predictors within the imputation, including categorical variables such as species, sex, and nominal classification. This is because the imputation model must include all variables to be used in the analysis model (Moons et al., 2006), although it is not of importance whether all variables used in the imputation model are used in the analysis model (White et al., 2011). Therefore the original categorical variables are reassigned to the imputed dataset of continuous variables, to avoid the possibility of misclassification of individuals.

MICE is implemented through the "mice" package in the R environment (R Core Team, 2015) using the "norm" function (MIPCA (regularised), imp:norm: package = norm). It is suggested that a sensible number of imputations should roughly equal the percentage of missing values (van Buuren, 2012), and as such 10 imputations are used here. The effectiveness of the imputation is assessed using a procrustean rotation plot (Clavel et al., 2014), and averaged imputation values are used for analysis.

Data scaling and shape PCA

Yamaguchi *et al*., (2013) found that major morphological differences between Javan and Balinese tigers are likely related to size differences through allometric scaling (Mazák, 2010), and it is therefore important to consider size and shape separately to differentiate their respective influences. The analysis undertaken here follows the methodology of (Baur & Leuenberger, 2011) to log-transform and center the data, so that measurements are independent of size (although not independent of allometry (Klingenberg, 2016)). By scaling the data in this way the effects of isometric size can be assessed in isolation from shape and allometry. The separation of isosize provides a useful metric for comparison between captive and wild specimens as skull size is commonly used as a proxy for body size (Cardini et al., 2007). Shape scaling has previously been previously been implemented in the cranial analysis of big cats (Khorozyan, 2014). A Principal Component Analysis (PCA) is performed upon

the scaled variables to create shape Principal Components (sPCs). Isometric size (isosize) of individuals is calculated as the geometric mean of all variables (Baur & Leuenberger, 2011; Khorozyan, 2014). I examine the relationships between the scaled variables, sPCs and isosize in relation to captivity status.

Data partitioning

Big cats exhibit strong sexual dimorphism (Smuts et al., 1978; O'Regan & Kitchener, 2005; Yamaguchi et al., 2013) and therefore sexes should be analysed separately when considering taxonomic relationships and environmental drivers of morphology. The data is therefore separately analysed by groupings of male and female lions and tigers. Geographic variation in both size and shape has been found in previous studies of both the lion and the tiger (Kitchener, 1999; Mazák & Groves, 2006; Barnett et al., 2008; Mazak, 2010; Mazák, 2010; Wilting et al., 2015), and so ignoring the geographic structure of the samples may obscure variation between captive and wild specimens. It may therefore be advantageous to further split the available data into groups of similar geographic origin. When analysing each subset of the data, the data is rescaled before re-performing the analyses. By splitting the data into male and female lions and tigers, and into groups of similar geographic origin, independent datasets are created to corroborate patterns found between captive and wild specimens. Owing to the well documented differences in foramen magnum height and cranial volume between captive and wild lions, which may be related to uniquely captive behaviours or conditions (Saragusty et al., 2014), PCAs are performed on data without these two variables to highlight other differences between captive and wild specimens of male and female lions and tigers, and of nominal classes.

T-Tests

Standard t-tests are used to highlight variables which are significantly different between captive and wild specimens. Due to the large number of measurements, a Bonferroni correction is applied by dividing the standard p-value of 0.05 by the number of variables tested. The standard 0.05 value also is displayed to show variables which may differ

significantly. By highlighting measurements which differ significantly, overall patterns of variation between captive and wild specimens are visualised.

Data hygiene

From the original 75 measurements made on each skull, 19 are removed due to high intra-observer measurement error found either through this study or from Barnett et al., (2008) (*Table S1*). Measurements of canines were problematic due to incompleteness in the specimens, breakage, wear rates with age, intra-observer error, and the difficulty in knowing where to anchor measurements, especially where the cemento-enamel junction was irregular. As a result I removed all measurements from subsequent analyses. From a total sample of 500 lions and 393 tigers, 69 juvenile and sub-adult lions and 62 sub-adult tigers are removed because of their incomplete development. In addition, 90 individuals with over 20% missing measurements, and 45 specimens that had missing mandibles were also removed to improve the imputation procedure. The effectiveness of the imputations is displayed using a procrustean rotation plot in *Figure S2* of the Supplementary Information. Following the imputation procedure, I further removed five specimens where the captivity status was unknown.

Results

The final dataset utilised here consists of 56 measurements of 621 specimens (*Table 1 and Table S1* in the Supplementary Information).

Table 1: A breakdown of the available specimens used within our analysis after the removal of individuals with very large numbers of incomplete measurements, juvenile and subadult specimens, and those whose captivity status was not recorded.

	Sex	Captive	Wild	Total
Lion	**Male**	33	151	184
	Female	17	143	160
Tiger	**Male**	36	112	148
	Female	33	96	129

It is apparent from *Table 1* that the dataset consists of considerably more wild specimens than captive specimens, especially for lions. The geographical origin of captive and wild

specimens is not random (*Figure 1*), and certain putative taxonomic groups are represented in greater or lesser numbers depending on captivity status. Captive tigers largely consist of specimens recorded as *P.t.altaica* (Amur tigers) and *P.t.sumatrae* (Sumatran tigers), which are also well represented by wild specimens. A large number of wild *P.t.sondaica* (Javan tigers) and *P.t.tigris* (Indian subcontinent) are available, however there are very few captive specimens from these groups. The lion dataset is overwhelmingly comprised of wild specimens recorded as *P.l.nubica* (East Africa), although there are very few captive specimens of this putative subspecies. The available data provide three levels of analysis. **1)** an analysis of all data together **2)** an analysis of four groupings (male and female lions and tigers) which provides four independent datasets for validation. **3)** an analysis of groupings from more similar geographical or taxonomic origin. Given the data available, captive and wild tigers from the putative groupings *P.t.sumatrae* and *P.t.altaica* are analysed separately. Due to the paucity of captive data in the lion, however, I examine the putative groups *P.l.senegalensis* and *P.l.persica* together, which despite their geographical separation, most probably represent members of the same subspecies (Kitchener et al., 2017).

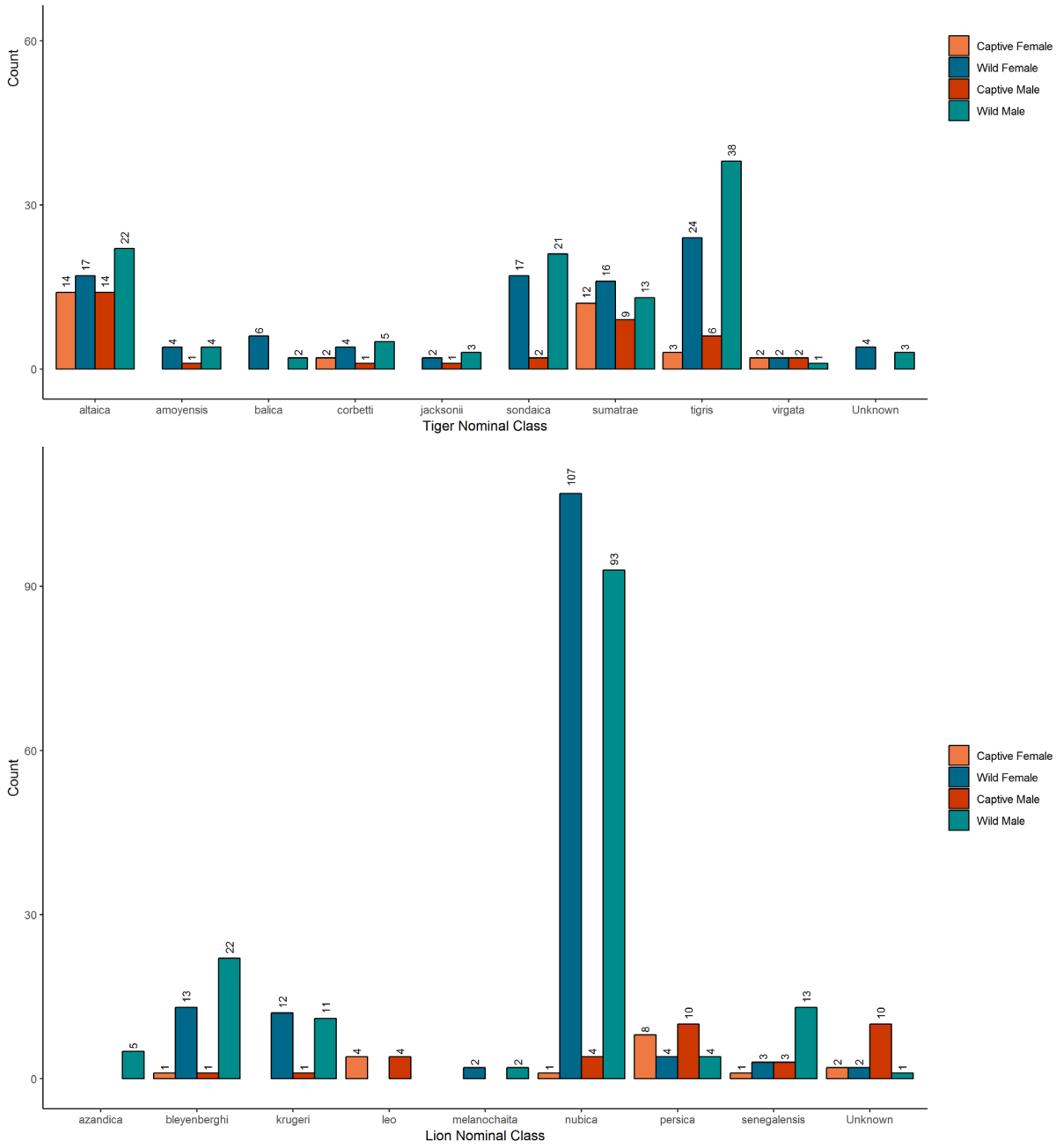


Figure 1: Available data for analysis grouped by sex, captivity status, and by recorded putative subspecies.

Initially both male and female lions and tigers are investigated together (*Figure 2*). There is clear size-related sexual dimorphism in both the lion and tiger, with males being larger. There is greater size overlap between males and females in the tiger than in the lion. Shape principal component 1 (sPC1) (25.3% contribution) distinguishes between the lion and the tiger with little overlap between each species of the same sex. sPC2 (22.7% contribution) separates males and females of both species although there is considerable overlap. There is some indication that captive individuals differ from wild individuals across sPC3 (6% contribution), although there is considerable overlap.

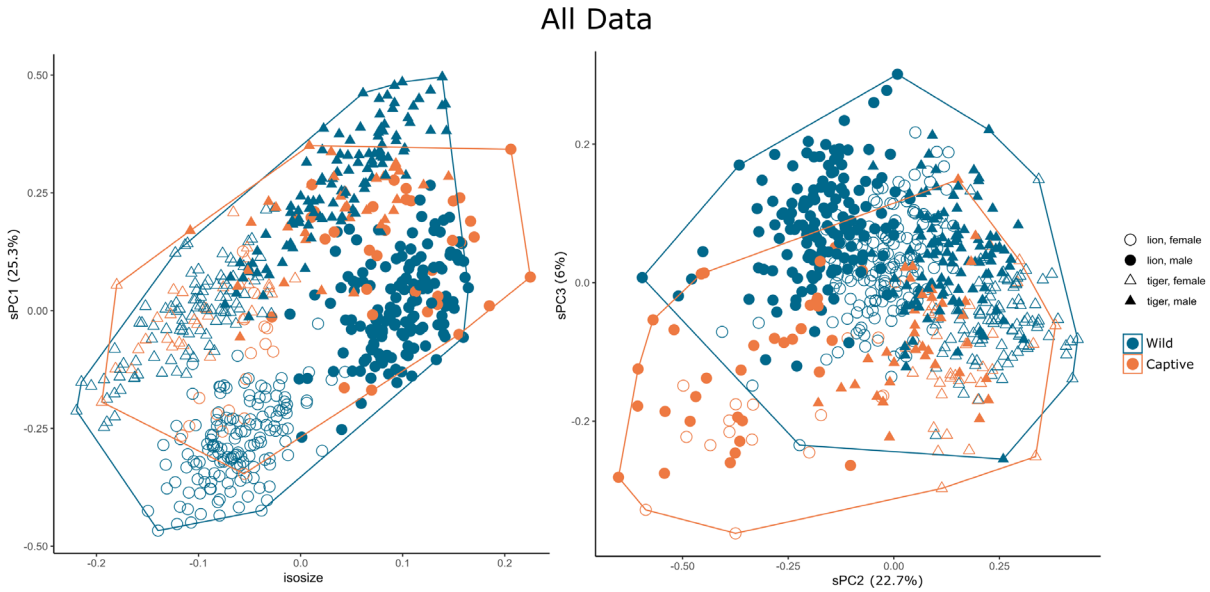


Figure 2: The relationship between captive and wild specimens by isosize and shape sPCs when male and female lions and tigers are analysed together.

Following the initial exploratory analysis of the data, foramen magnum height and cranial volume are removed from subsequent shape PCAs due to the very high contribution of these variables to the overall variance, and due to their known discriminatory power between captive and wild specimens of lions (Saragusty et al., 2014). Male and female lions and tigers are considered separately here, so that the variation between species and sex is removed from the analysis (*Figures 3+4*). Male and female lions show some differentiation by captivity status across sPC1 (14.9%+17.5% contributions respectively), and male and female tigers across sPC2 (13.6+13.6% contributions respectively). It is apparent that the putative classification may influence the analysis. For example most captive female lions are classed as *persica* (Asian lions), and although these are separated from wild *persica* specimens this may affect the clarity of the results. Given the large number of captive and wild specimens from *altaica* and *sumatrae* for the tiger, there is less chance of bias due to geographical origin. It is of interest that captive *altaica* and *sumatrae* group together across sPC2, away from their wild counterparts, despite their very different geographical origins, taxonomic status, and isometric size – this supports the notion that sPC2 shows variation determined by captivity status, yet there is still large overlap when the data are considered as a whole. It is therefore beneficial to separate out geographical groupings for further analysis of more similar specimens.

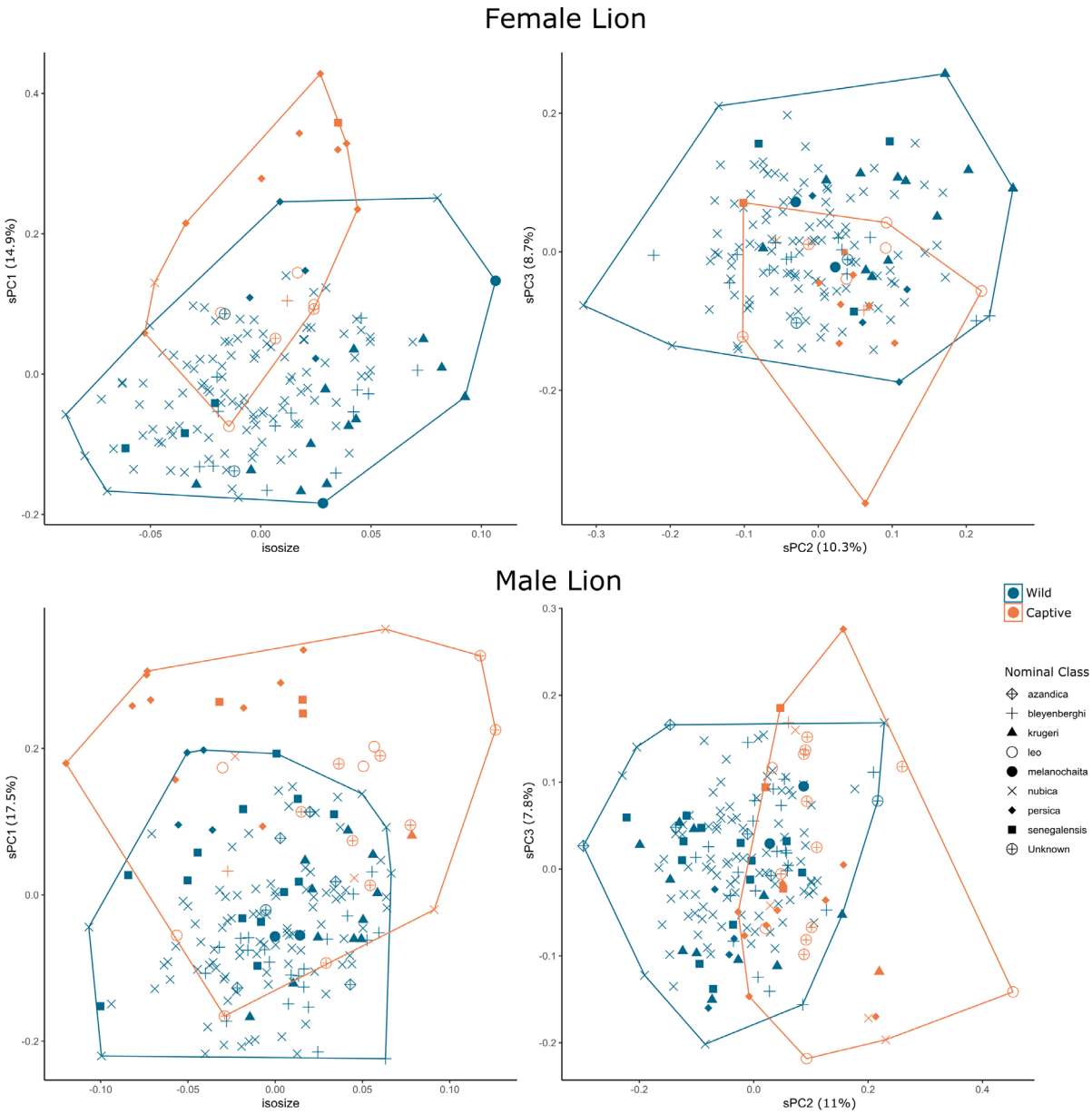


Figure 3: Captive and wild differentiation between captive and wild specimens by isosize and sPCs for female and male lions.

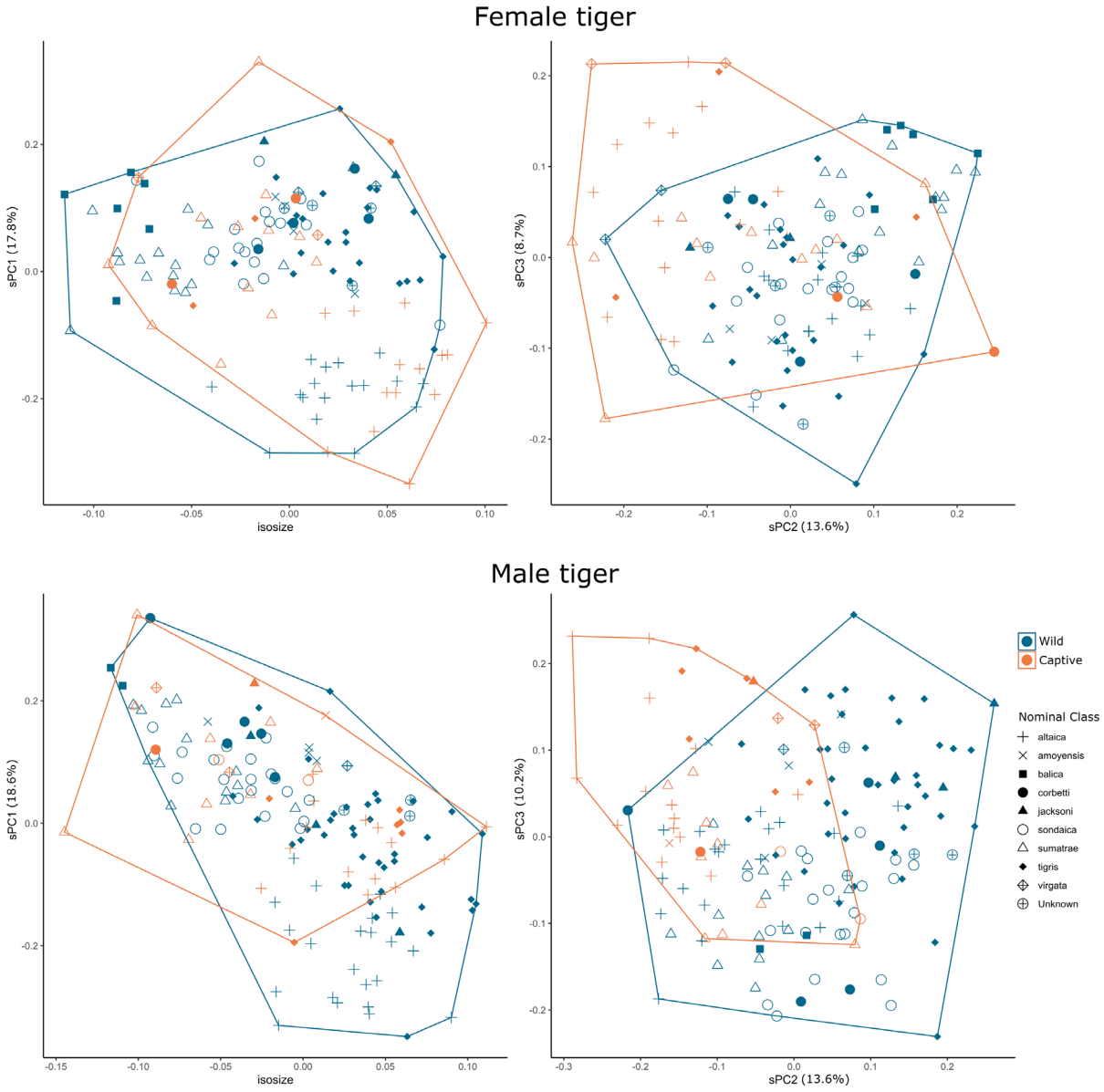


Figure 4: Captive and wild differentiation between captive and wild specimens by isosize and sPCs for female and male tigers.

Due to their discriminatory power by captivity status, the loadings of sPC1 for male and female lions, and sPC2 for male and female tigers are displayed in *Figure 5*. *Figures S3-S5 of the Supplementary Information* display loadings for all sPCs. Violin plots further highlight the differences between captive and wild specimens based on these sPCs. In sPC1 for the male and female lion, increases in measurements of the neurocranium are negatively correlated with measurements of the teeth, and certain measurements of the mandible such as *mandible height of the angular process*. The pattern is less clear in sPC2 for male and female tigers.

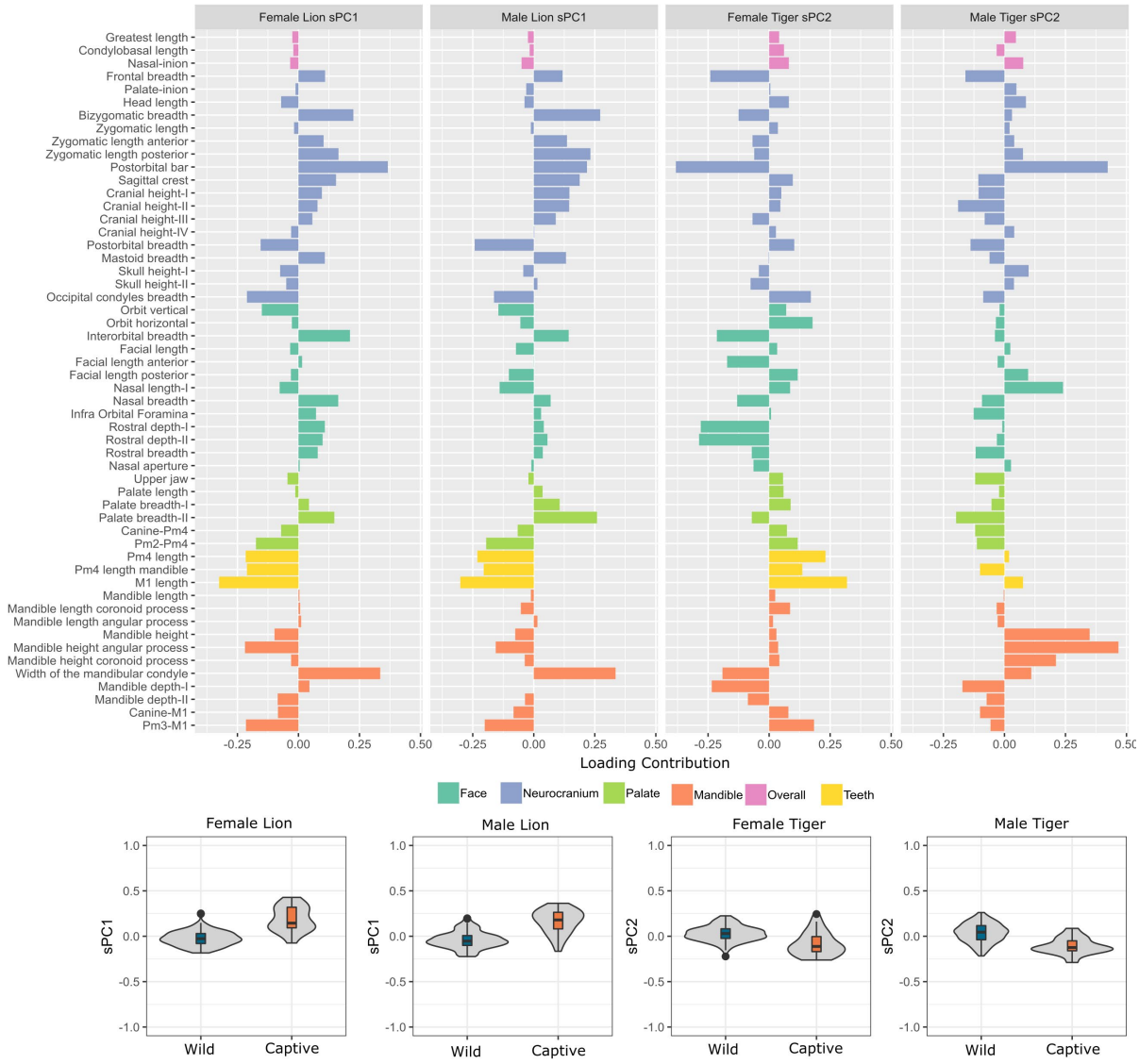


Figure 5: Loading contributions of the sPCs which visually discriminate between captive and wild lions and tigers determined by *Figures 3+4*. This constitutes sPC1 in lions and sPC2 in tigers. The distributions of captive and wild specimens are shown for each sPC using violin plots.

To further explore the patterns of variation between captive and wild specimens, box and whisker plots are used for each variable, separated by the area of the skull represented by the measurement (*Figures 6-10*). Statistical differences determined by t-tests (*Table S2 of the Supplementary Information*) are displayed by either orange (captive) or blue (wild) depending on which measurement mean is largest. Measurements of overall skull length (*Figure 6*) are not consistently affected by captivity status, which is concordant with plots showing no differentiation between captivity status by isosize. Because the variables are scaled by isometric size, large measurements of skull length exhibit low variance. *Figure 7*

shows that rostral depths and breadth measurements are generally larger in captive specimens. Anterior facial length may be larger in captive specimens whereas posterior facial length may be smaller. That nasal length is smaller in captive specimens supports the tentative finding that posterior facial length is also smaller.

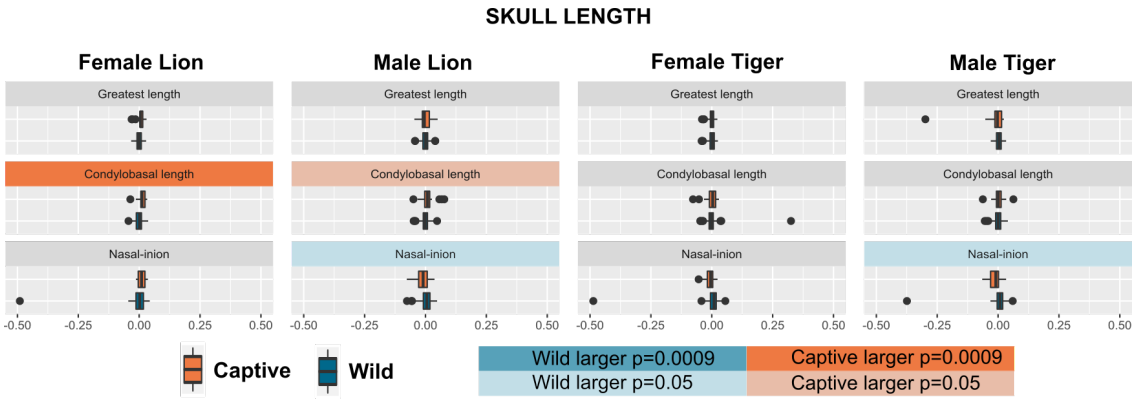


Figure 6: Variation of size independent (scaled) variables by captivity status for measurements representing skull length. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.** Measurements which differ significantly by captivity status are highlighted by which population mean is larger. Significance is determined using t-tests (*Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009.

Measurements of the neurocranium (*Figure 8*) show that whilst cranial volume is smaller in captive specimens, skull breadth and height measurements are generally larger, especially in the lion. Foramen magnum height and the nearby measurement of occipital condyles breadth are smaller in captive specimens, especially in the lion. Captivity status in the tiger does not appear to affect foramen magnum height, although there is large variation in this measurement in both captive and wild specimens. Consistent with captive skulls being broader, measurements of the palate tend to be broader, especially in lions which also exhibit longer palate length. Conversely, tooth lengths are generally reduced in captive specimens.

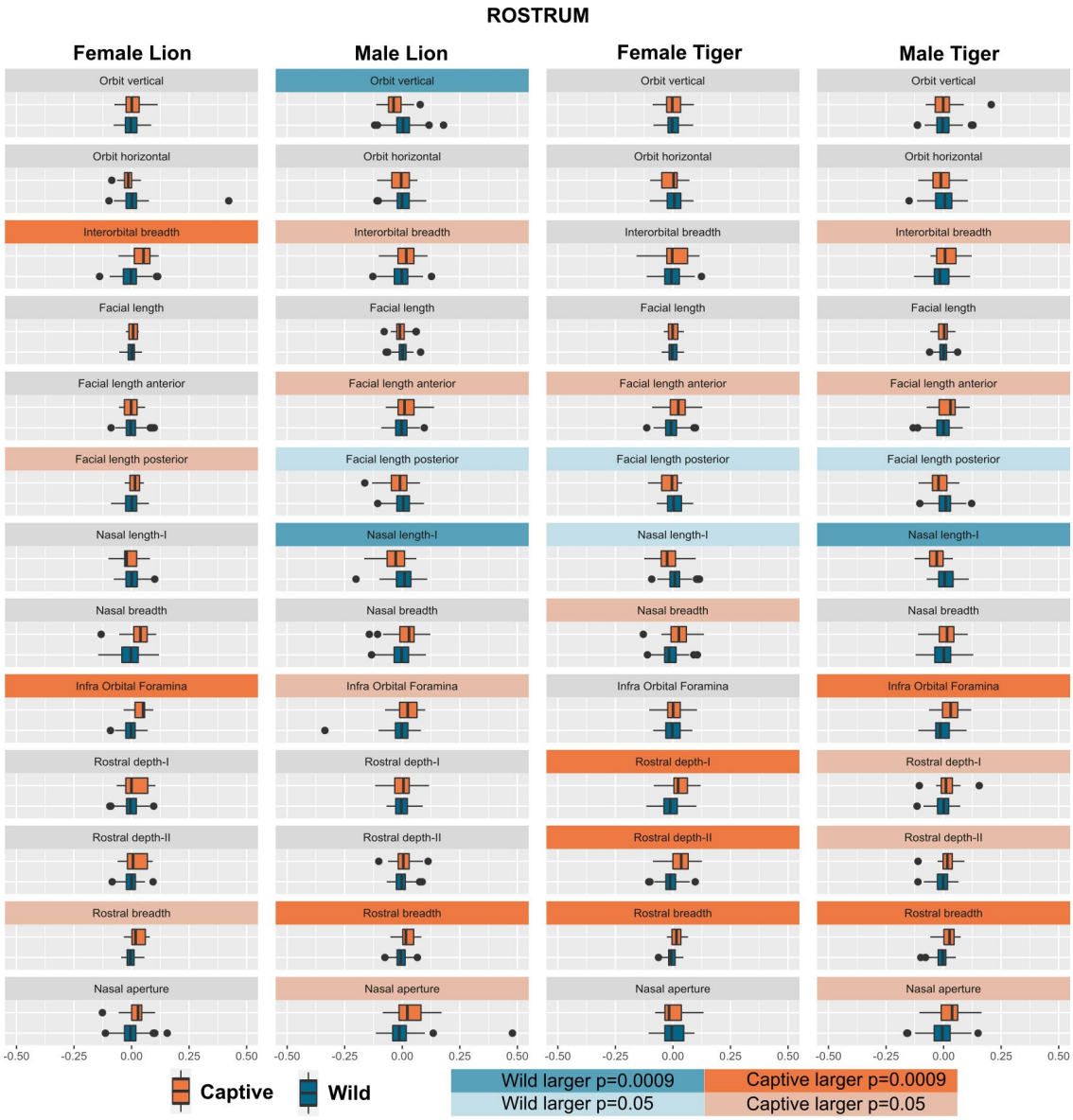


Figure 7: Variation of size independent (scaled) variables by captivity status for variables surrounding the rostrum. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.** Measurements which differ significantly by captivity status are highlighted by which population mean is larger. Significance is determined using t-tests (*Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009.

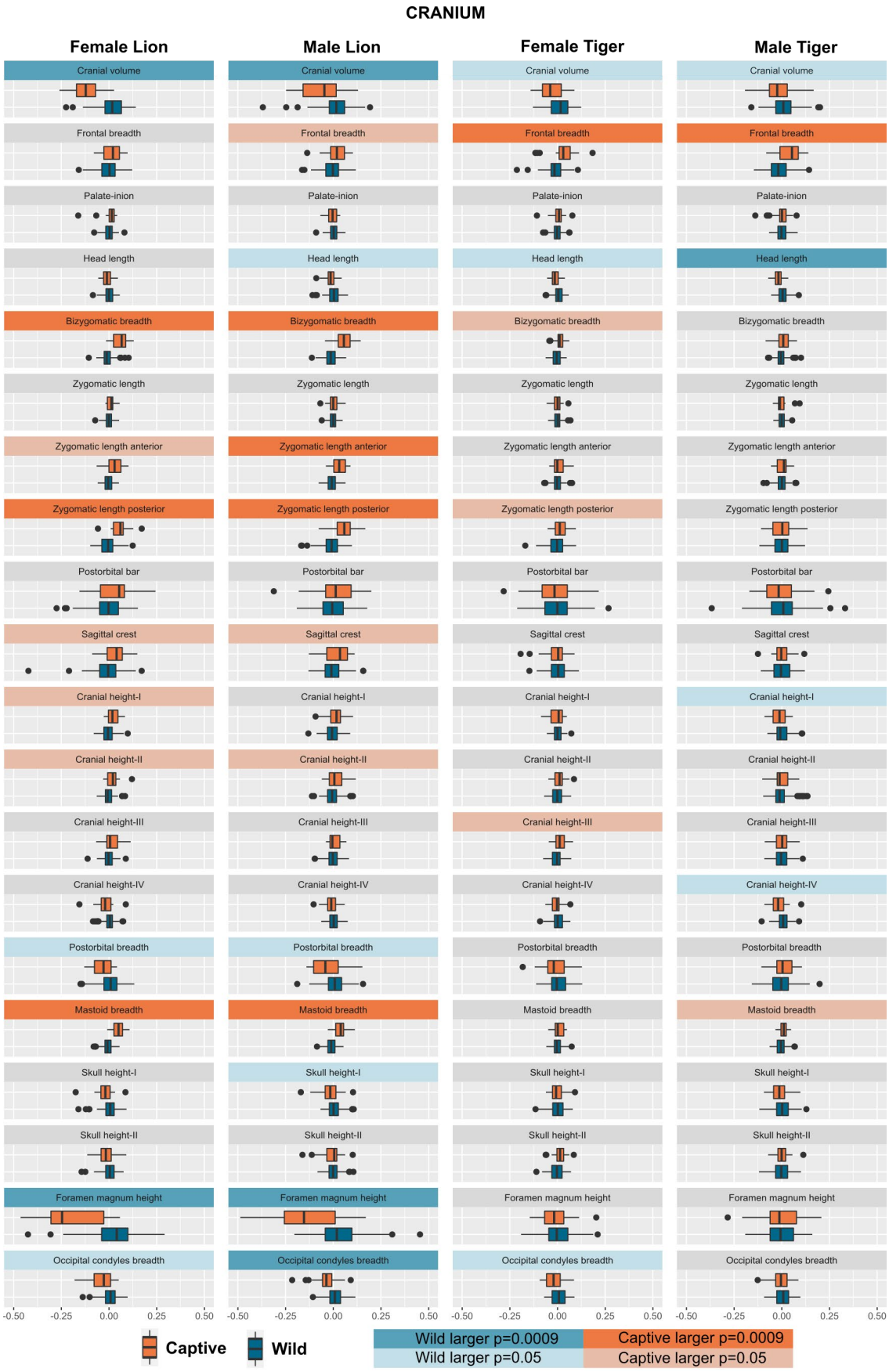


Figure 8: Variation of size independent (scaled) variables by captivity status for variables surrounding the cranium. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.** Measurements which differ significantly by captivity status are highlighted by which population mean is larger. Significance is determined using t-tests (*Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009.

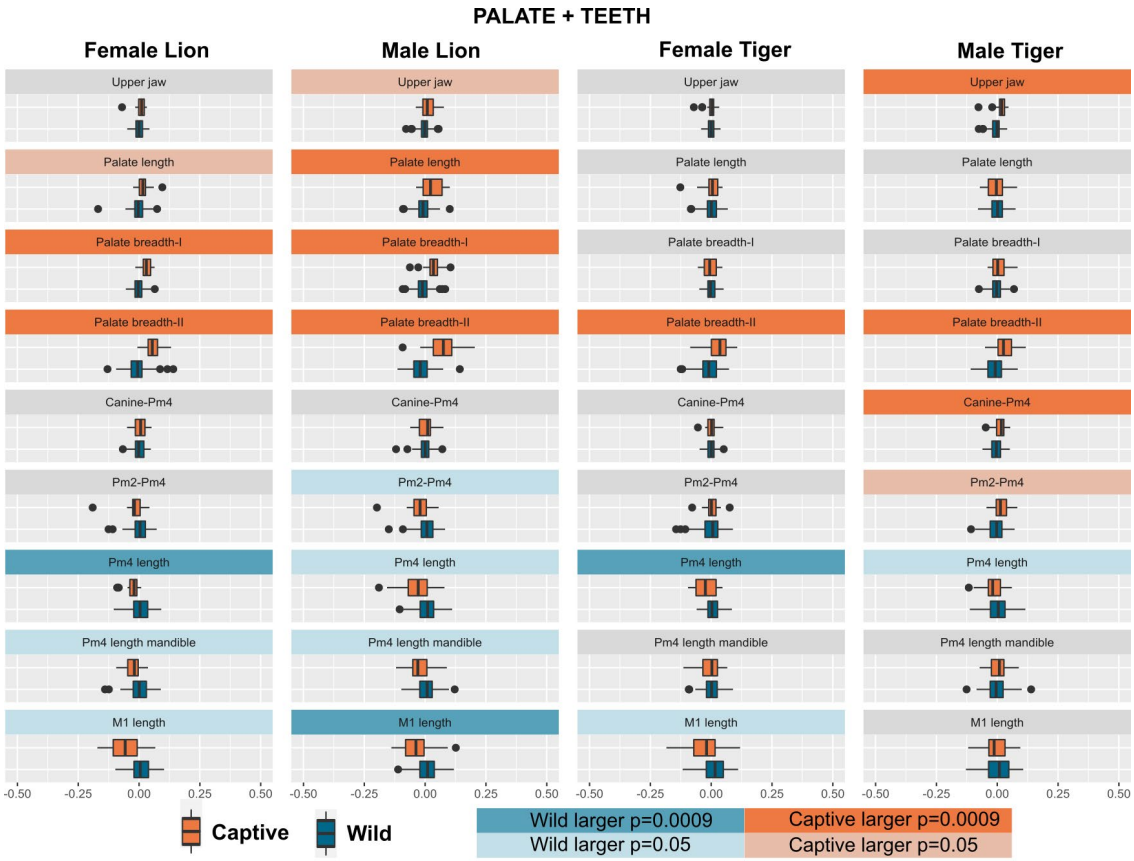


Figure 9: Variation of size independent (scaled) variables by captivity status for measurements of the palate and teeth. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.** Measurements which differ significantly by captivity status are highlighted by which population mean is larger. Significance is determined using t-tests (*Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009.

Figure 10 shows that whilst mandible length from the angular process, mandible depth-I and width of the mandibular condyles is generally larger in captivity, mandible height measurements are consistently smaller.

As well as showing the differences between captive and wild specimens *Figures 6-10* highlight measurements which do not change consistently or significantly by captivity status. This is apparent in measurements of overall skull length, measurements of the orbit, overall facial length, palate-inion, overall zygomatic length and measurements to both cranial height and skull height. The postorbital bar does not differentiate by captivity status, but is of interest due to its relatively large variance in both captive and wild specimens (*Figure 8*).

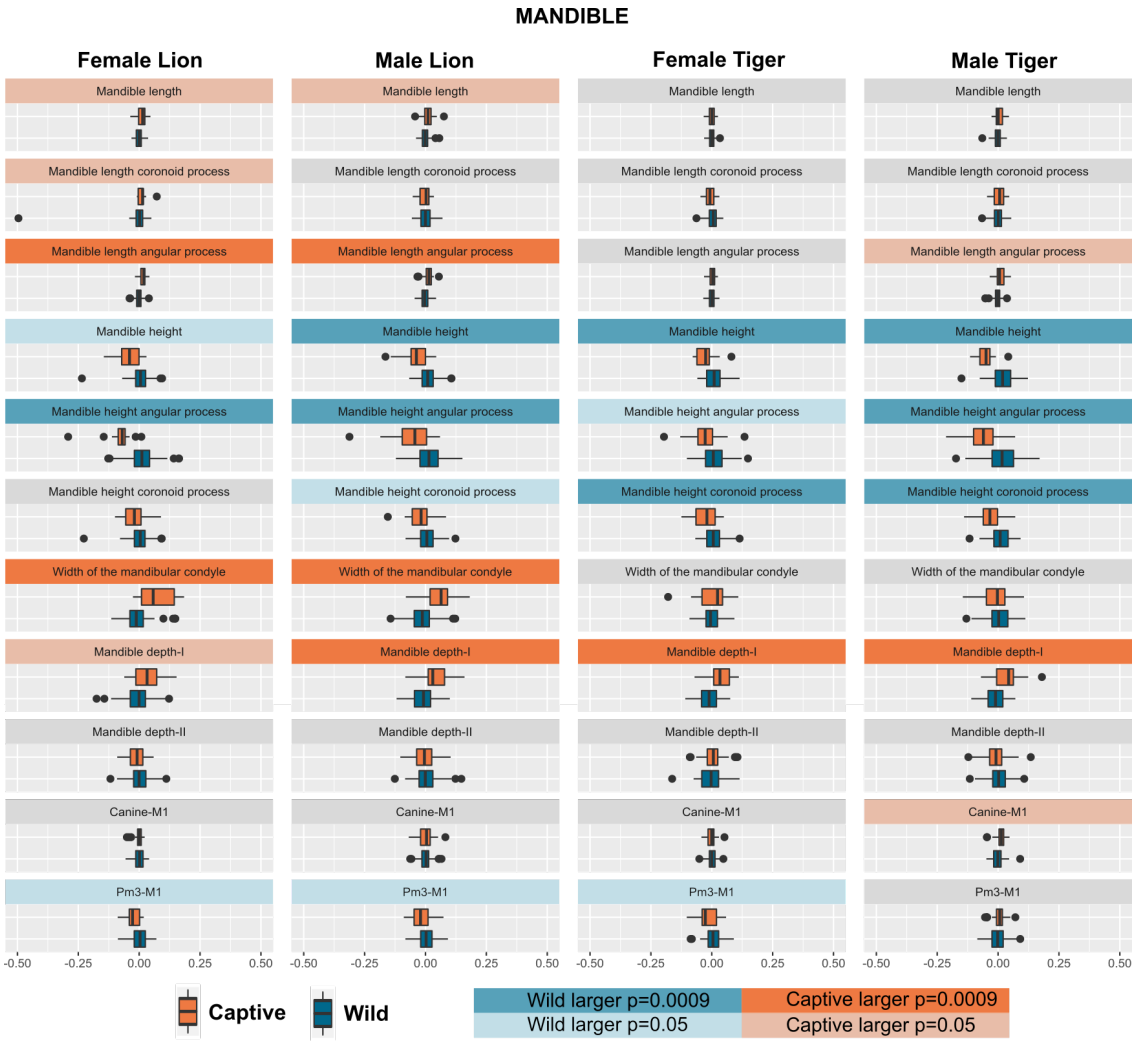


Figure 10: Variation of size independent (scaled) variables by captivity status for measurements of the mandible. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.** Measurements which differ significantly by captivity status are highlighted by which population mean is larger. Significance is determined using t-tests (*Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009.

On separating the data into male and female lions and tigers, the strong influence of species and sex is removed from the analysis, yet it is apparent that there is variation in skull dimensions dependent on geographical origin, represented by the recorded putative subspecies of each specimen (*Figures 3+4*). *Figure 11* shows the separate sPCA and isosize for male and female tigers recorded as *altaica*, and as *sumatrae* and of lions recorded as *persica* and *senegalensis*. There is no difference in size between captive and wild specimens at this scale of analysis (or found at coarser scales of investigation). Captivity status is distinguishable between specimens across sPC1 in female and male tigers recorded as *altaica* (23+25% contribution respectively) with very low overlap. sPC1 separates female tigers recorded as *sumatrae* (34.7% contribution) and sPC2 for male tigers recorded as *sumatrae* (19.9% contribution), although there is greater overlap between captive and wild specimens of *sumatrae*. Captive and wild specimens of the lion (*persica* + *senegalensis*) are distinguishable (with some overlap) for males and female across sPC1 and sPC2, however it is apparent that this is at least in part due to geographical origin rather than captivity status.

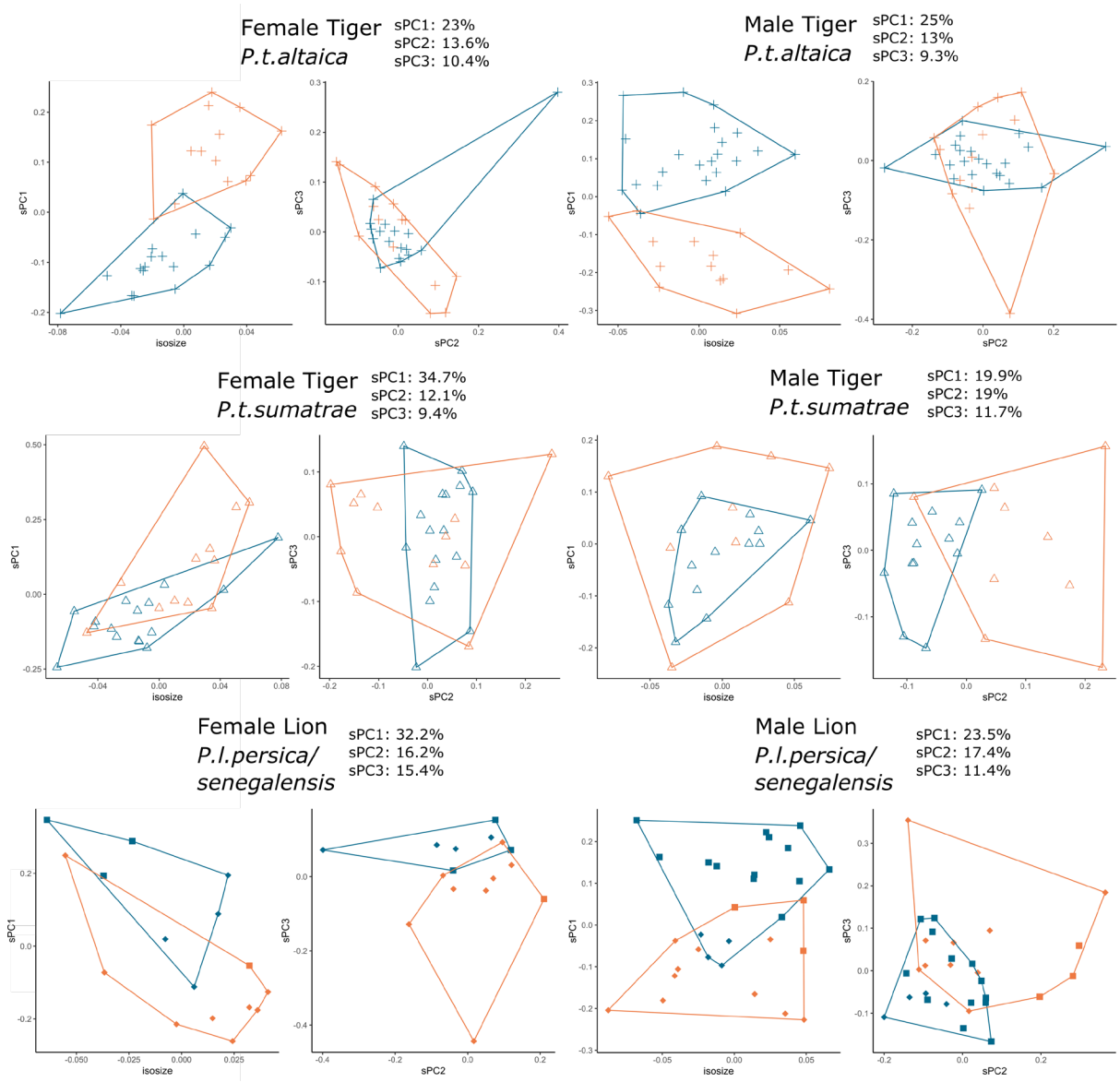


Figure 11: Captive and wild differentiation between captive and wild specimens by isosize and sPCs for nominal classes of the lion and the tiger.

Measurements differ between captive and wild specimens in the same general patterns for each nominal class as found when analysed by male and female lions and tigers (*Figures S6-S11 of the Supplementary Information*). One exception to this is the significant differentiation between sagittal crest length and cranial heights of male Amur tigers (*P.t.altaica*), which do not separate captive from wild specimens for any other grouping (*Figure 12*). Captive male Amur tigers have significantly smaller sagittal crest lengths, and cranial heights measurements (which account for sagittal crest height). There is some visual suggestion that this pattern also occurs in female Amur tigers, however it is generally not significant.

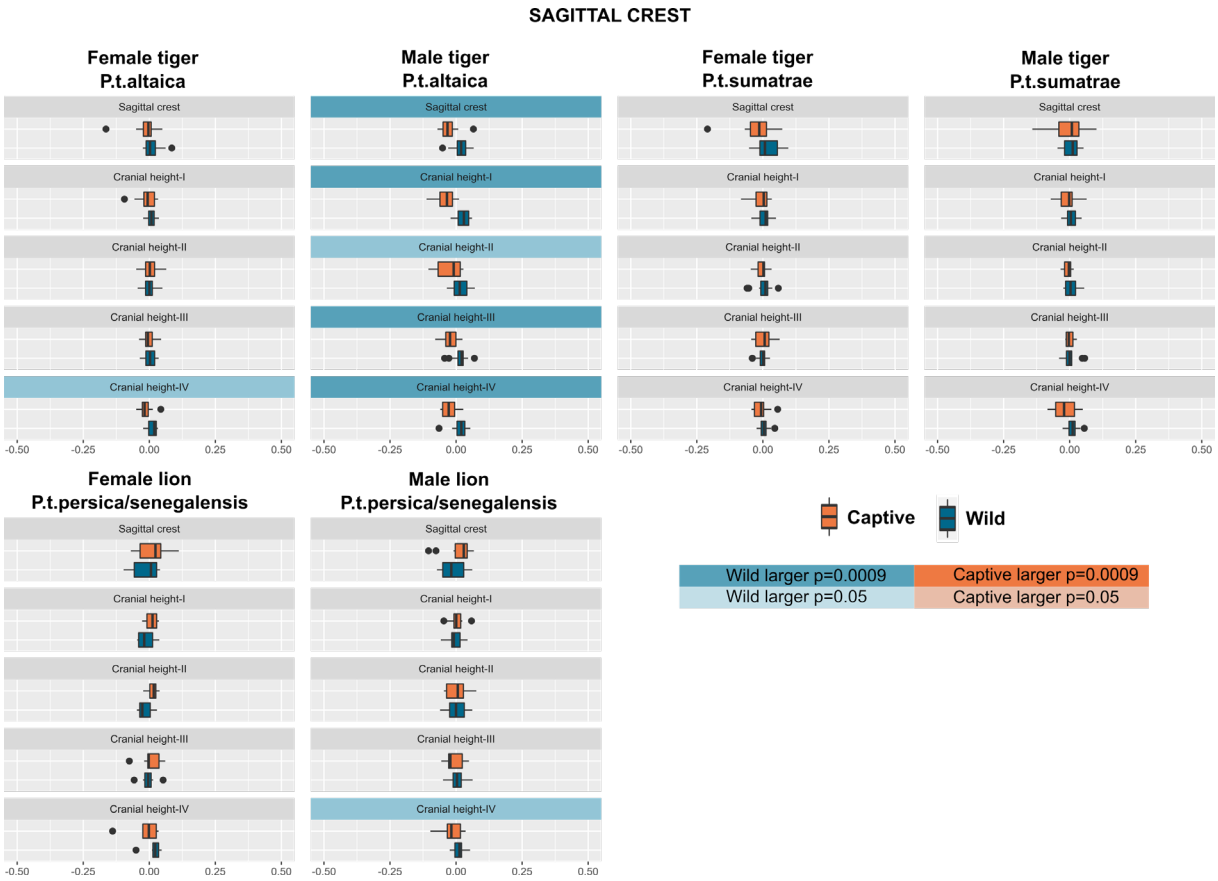


Figure 12: Variation of size independent (scaled) variables by captivity status for measurements of the sagittal crest when the data is split by nominal grouping. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.** Measurements which differ significantly by captivity status are highlighted by which population mean is larger. Significance is determined using t-tests (*Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009.

Discussion

Data and methodology

The new analyses reported here create novel opportunities to explore variation in morphological characteristics using an exceptionally large number of specimens and linear measurements. The very large number of measurements taken from each specimen has enabled the removal of incomplete or unreliable variables, whilst still retaining the overall character of skull shape and size. By taking multiple, similar measurements, the results also show groups of measurements around functional skull areas, which vary in similar patterns, and further support these analyses. Whilst the field of morphometrics has largely progressed towards geometric morphometrics, the results show clear parallels with the results derived from landmark data (Hartstone-Rose et al., 2014), and highlight the continuing relevance of simpler linear techniques.

This analysis accounts for specimens which have died in captivity, but it is often not apparent whether specimens were captured from the wild, and at what stage of development age this may have occurred. Whilst modern captive specimens tend to be born in captivity, adult specimens removed from the wild that have died in captivity could be more likely to group with wild specimens than individuals born in captivity. However, the clear differentiation between captive and wild specimens when analysed by similar taxonomic groups shows that this has not greatly affected the results reported here.

Broad data patterns

When the data are analysed together, I find that species (sPC1) and sex (sPC2) account for similar levels of variation, and that males are consistently larger than females in both species. sPC1 also has a strong role in separating sex of both species, which is likely due to allometric scaling given the size disparity of males and females. sPC3 visually accounts for differentiation between captive and wild specimens, although there is considerable overlap and this component accounts for only 6% of the variation compared to ~20% for sPC1 and ~20% for sPC2. Whilst previous studies have found that captivity status is nearly twice as discriminating as sex in craniometric studies of the lion and tiger (Hartstone-Rose et al.,

2014), the results presented here find sex is over three times more discriminatory than captivity status. This difference may be due to the widespread use of ground meat diets in captive North American institutions, which lack the mechanical properties of a natural diet, and are the source of captive specimens from previous analyses (McPhee, 2002; Hartstone-Rose et al., 2014), although it may also be due to the removal of Principal Component 1 from the previous analysis. This step removes the allometric scaling influence which differentiates male and female specimens based on size. The majority of captive specimens from this study originate from European zoos which typically use whole or part carcass feeds (Bond & Lindburg, 1990), which may better replicate the mechanical properties of a wild diet upon the skull. Even for older specimens from the turn of the 20th century within this analysis, when captive environments were comparatively barren and unnatural, carcass feeding was used (Hollister, 1917).

Captive and wild differentiation between male and female lions and tigers

When specimens of similar taxonomic status are analysed, the differentiation between captive and wild specimens is markedly more apparent, often accounting for the first principal component, with very little overlap. Whilst the use of t-tests has highlighted significant individual measurements, understanding the differences between captive and wild specimens is best approached by looking at how measurements change in relation to each other in response to captivity status. This approach prevents the over-interpretation of the effects of individual measurements and provides a more holistic interpretation of skull differentiation. The wider skull dimensions and shorter mandible heights of captive specimens has been found in previous studies and likely relate to the mechanical properties of captive diets (Hollister, 1917; Hartstone-Rose et al., 2014). The primary jaw muscles are the temporalis and masseter (Sicuro & Oliveira, 2011). The masseter muscles originate at the zygomatic arch (Hartstone-Rose et al., 2014) and insert on the mandible. The masseter superficialis is related to the angular process and posterior mandible (Turnbull, 1970). A high coronoid process improves the mechanical leverage of the temporalis muscle (Sicuro & Oliveira, 2011) and it is therefore conceivable that variation in the use of the temporalis muscle may affect the height of the coronoid process during development. Repeated higher forces upon the structures surrounding these muscles, and subject to high leverage from bite forces in wild specimens has likely caused the disparity between captive and wild lions and tigers.

Whilst patterns between captive and wild populations are largely in agreement across male and female lions and tigers, there are patterns between partitions of certain geographical groups and sexes which are not apparent in other groups. Differentiation between captive and wild Amur tigers by sagittal crest height and length has been suggested by previous studies, which propose that a flattening of the sagittal crest is due to a reduction in the action of the temporalis (Duckler, 1998). The lesser differentiation by captivity status on sagittal crest height or length in female Amur tigers is likely due to their generally less pronounced sagittal crest for a given body size regardless of captivity status. The pattern is not seen in either male or female Sumatran tigers, or in any grouping of the lion. Variation in the sagittal crest has been observed in large wild male lions (Christiansen, 2008), and between wild Amur/Caspian and other mainland tigers (Mazák, 1996; Mazak, 2010) and is therefore of potential significance in further analysis of wild specimens.

The lack of overlap in the principal component analyses of geographically and taxonomically similar specimens shows that the effects of a captive lifestyle are still apparent, despite many European zoos utilising enrichment practices and carcass feeding, which better replicate natural stresses on the skull and reduce stereotypic behaviours (McPhee, 2002; Skibiel et al., 2007). Beyond the mechanical effect of feeding itself, it is possible that the intensity of prey immobilization, killing strategy, carcass dragging and general manipulation in the wild is not well replicated in captivity.

Effects other than the mechanical properties of diet may have played a role in differentiating by captivity status. The reduction in foramen magnum height in captive lions (foramen magnum stenosis) has been attributed to vitamin deficiencies (Saragusty et al., 2014), and is unlikely to be due to the mechanical properties of diet (Hartstone-Rose et al., 2014). Alternatively, stereotypic behaviours in captivity, for example, excessive grooming, may cause variation between captive and wild samples that is independent from masticatory stress (Duckler, 1998). With few notable exceptions, animals tend to live longer in captivity (O'Regan & Kitchener, 2005). Tooth measurements are affected by the abrasive action of different diets (Hartstone-Rose et al., 2014) and big cats rely heavily on their canines and carnassials when feeding in the wild, but to a far lesser extent in captivity (Zuccarelli, 2004), especially if fed on a processed diet. The increased potential for tooth wear in wild specimens may be offset by a shorter lifespan which may explain the generally smaller tooth

measurements found in captivity reported here. It is of interest that the relatively narrower skulls of wild lions and tigers have an increased cranial volume. Brain size increases in mammals in more enriched environments, and captive-bred mammals from multiple species have been found to have smaller brains (O'Regan & Kitchener, 2005). While it has been suggested that the smaller cranial volume of captive lions is pathological and does not have application to the understanding of normal (wild) skulls (Howell, 1917), it could also be bound up with the development of jaw musculature impacting skull development as well as the complexity of the environment impacting brain development. An assessment of the variation in cranial volume in wild specimens may determine the pathological status of cranial volume in captivity.

Skull metrics unaffected by captivity status

The lack of size differentiation between captive and wild specimens is consistent with previous studies (Hartstone-Rose et al., 2014). Skull size between wild felids has not been found to correlate with variations in jaw mechanics (Sicuro & Oliveira, 2011). Measurements that are strongly associated with overall size are not significantly different between captive and wild individuals. Beyond measures of overall size, the results highlight measurements that are not consistently affected by captivity status across all regions of the skull. It is likely that these measurements are less affected by bite forces, such as measurements of the orbit, and measurements of skull height. Whilst cranial height measurements do not consistently differ between captive and wild specimens, the variation found in male Amur tigers suggests that these measurements are phenotypically plastic in specimens predisposed to large sagittal crests.

As found previously (Saragusty et al., 2014), foramen magnum height and cranial volume vary considerably between captive and wild lions, but not in tigers. Whilst foramen magnum height does not discriminate between captive and wild tigers, there is relatively large variation of this measurement in tigers and it may be relevant to variation between populations in the wild. The results show that occipital condyles breadth is also narrower in captive lions, but not tigers, which is likely related to the causes of reduced foramen magnum height. Further investigation into the variation in foramen magnum height in wild populations may answer whether foramen magnum stenosis has a genetic basis as proposed by Saragusty

et al., (2014). Hollister (1917) found captive lions to have a thickened malar, which roughly corresponds to the measurement of the postorbital bar described here. Whilst the results here show very large variance in the postorbital bar, as shown in the loadings of sPCs, it does not show any statistical difference by captivity status (*Table S2 in the Supplementary Information*). A low mean coefficient of variation shows that this measurement is robust to error (*Table S1 in the Supplementary Information*) and so variation is unlikely to have come from poor measurement accuracy. It is unlikely that the postorbital bar is related to the evolutionary history of an individual as it is shown to have high variance even when similar groups are assessed (*Figures 6+7*).

Conclusion

The new results presented here show that the skulls of male and female lions and tigers differ when subjected to a captive versus wild environment. Whilst overall skull size does not differ between captive and wild specimens, an overall pattern of wider skull dimensions and shorter mandible heights is found in captivity. The results highlight the importance of comparing specimens from comparable taxonomic origins, as morphological differences across the geographic range of each species can obscure patterns between captive and wild specimens. When similar geographic groupings are analysed there is minimal overlap between captive and wild specimens within certain principal component analyses of shape. Whilst differences between captive and wild specimens are attributed to diet, the prevalence of carcass feeding in the predominantly European institutions analysed, and lack of overlap between captive and wild specimens, suggests that the mechanics of diet in the wild are not entirely replicated by carcass feeding in captivity. Differentiation between wild and captive Amur tigers by sagittal crest height and length is unique, and the known differentiation of Amur tigers from other mainland tigers by sagittal crest length, suggests that phenotypic plasticity could be a driver of differentiation. Variation of the sagittal crest is therefore a significant consideration in the future analysis of wild specimens. Cranial volume and foramen magnum height differentiate between captivity status in lions but not in tigers, although there is high variation in these measurements in both species. Further analysis of wild specimens will shed light on the influence of these measurements on the tiger, and also the suggested pathological status of these structures in captive lions.

This chapter highlights the potential for phenotypic plasticity in the skull of the lion and tiger, and suggests that the operation of jaw mechanics best explains the patterns of change. It provides crucial new information to the following analysis of variation within wild specimens and understanding of the manifestation of evolutionary histories, and raises questions about the nature of captivity related malformations, which may be resolved by an understanding of their variation in the wild.

Supplementary Information

Table S1: Measurements taken corresponding to the subsequent definition of points, definition of measurements, and *Figure S1*. Measurements with a mean coefficient of variation > 1, identified either from this study, or from (Barnett et al., 2008) are removed from imputation and analysis (**identified in bold**). Cranial volume is kept due to its known discriminatory power between captive and wild lions (Saragusty et al., 2014), and all canine measurements are removed due to apparent uncertainties identified during the measurement process.

Continuous Measurements	**Mean Coefficient of variation**	**Coefficient of variation** (Barnett et al., 2008)
Cranial volume (ml)	1.00 (± 0.305)	0.35 (± 0.084)
Frontal breadth [21]	0.05 (± 0.016)	0.08 (± 0.021)
Greatest length [A-B]	0.31 (± 0.193)	0.04 (± 0.012)
Condylobasal length [A-C]	0.13 (± 0.051)	0.03 (± 0.002)
Palate-inion [V-B]	0.20 (± 0.014)	0.14 (± 0.039)
Nasal-inion [E-B]	0.04 (± 0.015)	0.14 (± 0.035)
Facial length [A-G]	0.12 (± 0.045)	0.17 (± 0.034)
Head length [G-B]	0.07 (± 0.017)	0.08 (± 0.015)
Bizygomatic breadth [24]	0.03 (± 0.01)	0.04 (± 0.009)
Zygomatic length [K-M]	0.29 (± 0.116)	0.11 (± 0.028)
Zygomatic length anterior [K-L]	0.37 (± 0.066)	0.3 (± 0.048)
Zygomatic length posterior [L-M]	0.44 (± 0.08)	0.31 (± 0.064)
Orbit vertical [4]	0.24 (± 0.074)	0.5 (± 0.195)
Orbit horizontal [3]	0.55 (± 0.142)	0.94 (± 0.198)
Postorbital bar [2]	0.34 (± 0.128)	0.28 (± 0.043)
Facial length anterior [A-E]	0.6 (± 0.152)	0.59 (± 0.085)
Facial length posterior [E-G]	0.12 (± 0.019)	0.73 (± 0.166)
Sagittal crest [H-B]	0.25 (± 0.067)	0.23 (± 0.064)
Cranial height-I [N-H]	0.5 (± 0.124)	0.19 (± 0.027)
Cranial height-II [N-H']	0.46 (± 0.116)	0.4 (± 0.116)
Cranial height-III [N-H"]	0.3 (± 0.045)	0.33 (± 0.078)
Cranial height -IV [N-B]	0.18 (± 0.067)	0.34 (± 0.049)
Interorbital breadth [20]	0.27 (± 0.203)	0.1 (± 0.016)
Postorbital breadth [22]	0.17 (± 0.062)	0.15 (± 0.073)
Nasal length-I [D-F]	0.34 (± 0.091)	0.33 (± 0.144)
Nasal length-II [S-F]	**1.23 (± 0.251)**	0.42 (± 0.202)
Nasal breadth [D-D]	0.64 (± 0.144)	0.57 (± 0.11)
Breadth between infra orbital foramina [19]	0.25 (± 0.071)	0.17 (± 0.055)
Rostral depth-I [1]	0.62 (± 0.177)	0.75 (± 0.14)
Rostral depth-II [E - most posterior end of canine alveolus]	0.45 (± 0.05)	0.53 (± 0.057)
Rostral breadth [17]	0.13 (± 0.025)	0.16 (± 0.036)
Nasal aperture [18]	0.53 (± 0.071)	0.8 (± 0.448)
Upper jaw [A-U]	0.12 (± 0.029)	0.1 (± 0.018)
Palate length [T-V]	0.27 (± 0.065)	0.14 (± 0.041)
Palate breadth-I [29]	0.22 (± 0.04)	0.14 (± 0.034)

Palate breadth-II [28]	0.47 (± 0.103)	0.16 (± 0.045)
Canine - Pm^4 (alveolus - alveolus)	0.42 (± 0.116)	0.46 (± 0.136)
Pm^2 - Pm^4 (alveolus - alveolus)	0.43 (± 0.153)	0.25 (± 0.129)
Upper canine height [5]	**2.76 (± 0.717)**	0.36 (± 0.18)
Upper canine diameter antero-posterior [25]	0.69 (± 0.274)	0.53 (± 0.16)
Upper canine diameter medio-lateral [26]	0.88 (± 0.306)	0.57 (± 0.171)
Upper canine alveolus diameter antero-posterior	0.99 (± 0.49)	1 (± 0.233)
Upper canine alveolus diameter medio-lateral	**1.77 (± 0.36)**	2.02 (± 0.702)
Pm^4 length [6]	0.68 (± 0.378)	0.18 (± 0.04)
Pm^4 breadth-I [27]	**1.77 (± 1.01)**	0.83 (± 0.153)
Pm^4 breadth-II [27]	**1.78 (± 0.836)**	0.37 (± 0.092)
Mastoid breadth [23]	0.03 (± 0.01)	0.04 (± 0.009)
Skull height-I [W-B]	0.23 (± 0.038)	0.79 (± 0.41)
Skull height-II [7]	0.1 (± 0.028)	0.55 (± 0.32)
Foramen magnum breadth	**1.78 (± 1.139)**	0.16 (± 0.022)
Foramen magnum height [greatest distance: usually oblique]	0.5 (± 0.155)	0.65 (± 0.188)
Occipital condyles breadth [33]	0.04 (± 0.008)	0.08 (± 0.043)
Tympanic bulla length [31]	**1.58 (± 0.923)**	0.79 (± 0.137)
Tympanic bulla breadth-I [30]	0.89 (± 0.401)	**1.42 (± 0.559)**
Tympanic bulla breadth-II [32]	**2.22 (± 0.661)**	**2.96 (± 0.269)**
Mandible length [O-Q]	0.08 (± 0.031)	0.18 (± 0.042)
Mandible length coronoid process [O-Q']	0.05 (± 0.023)	0.19 (± 0.058)
Mandible length angular process [O-Q'']	0.1 (± 0.052)	0.07 (± 0.019)
Mandible height [13]	0.34 (± 0.152)	0.23 (± 0.069)
Mandible height angular process [14]	0.91 (± 0.344)	0.57 (± 0.182)
Mandible height coronoid process [15]	0.35 (± 0.126)	0.16 (± 0.04)
Maximum width of the mandibular condyle [16]	0.09 (± 0.023)	0.08 (± 0.02)
Mandible depth-I [11]	0.6 (± 0.176)	0.51 (± 0.237)
Mandible depth-II [12]	0.41 (± 0.262)	0.2 (± 0.043)
Canine - M^1 (alveolus - alveolus)	0.26 (± 0.036)	0.27 (± 0.088)
Pm^3 - M^1 (alveolus - alveolus)	0.52 (± 0.079)	0.3 (± 0.077)
Lower canine height [8]	0.45 (± 0.075)	**1.33 (± 0.463)**
Lower canine diameter antero-posterior	0.68 (± 0.269)	0.78 (± 0.222)
Lower canine diameter medio-lateral	**1.33 (± 0.222)**	1.56 (± 0.29)
Lower canine alveolus diameter antero-posterior	**1.11 (± 0.393)**	**1.03 (± 0.189)**
Lower canine alveolus diameter medio-lateral	**2.17 (± 0.269)**	**1.72 (± 0.674)**
Pm^4 length	0.71 (± 0.108)	0.13 (± 0.022)
Pm^4 breadth (largest breadth usually towards the posterior end	**2.06 (± 0.302)**	0.54 (± 0.178)

M^1 length [9]	0.48 (± 0.265)	0.95 (± 0.342)
M^1 breadth (largest breadth usually around the middle)	**1.97 (± 0.744)**	0.4 (± 0.117)

Definition for points (Barnett et al., 2008):

A. Prosthion: the most anterior point of the skull
B. Inion: the most posterior point of the skull
C. The line connecting the most posterior points of the occipital condyles
D. The most anterior points of the nasals
E. The highest points on the vaults of the anterior ends of the dorsal parts of the nasals
F. The most posterior point of the inter nasal suture
G. The point where the line connecting the most outer points of the postorbital process of the frontal meets the mesion
H. Bregma: where the coronal suture meets sagittal suture (if the sagittal crest is very well developed, use the place where the coronal suture reaches the top of the sagittal crest in the mesion)
H'. One third distance point between bregma and inion
H''. Two third distance point between bregma and inion
I. Vertical lines including the most outer points of the alveoli of I^3s
J. The most anterior point of alveolus of the upper canine
K. The most dorsal point of infraorbital foramen (in case if there are more than one foramen, the most dorsal point of the foramina)
L. The most outer point of the zygomatic arch (usually just above the malar - temporal suture)
M. A point where a vertical section including the most dorsal point of the auditory meatus cuts the outer curve of the zygomatic process of the temporal
N. The most dorsal point of the auditory meatus
O. Pogonion: the most anterior point of mandible on the inter mandible suture
P. The most ventral point around the angular process. If it is not obvious, use the point where the extended line of the middle line of inferior notch crosses the ventral edge of angular process as being shown in *Figure S1*. This may sound difficult, but in practice there is little problem and a subtle difference of the position of "P" does not seem to affect the result.
Q. The point where the extended line of the ventral end of superior notch crosses the posterior edge of condyle (approximately the middle of condyle)
Q'. The furthest point on the coronoid process from the pogonion
Q". The furthest point on the angular process from the pogonion
R. The line connecting the highest points on the vaults of the anterior ends of dorsal parts of nasals
S. The most anterior point of the inter nasal suture. Often the inter nasal suture of some skulls may be slightly opened towards the anterior end, and it may be difficult to assess where "S" is. In such case, ignore the part where the inner lines of the nasals forms a sallow angle (almost parallel) to the mesion, and find the point where the angle starts to change.
T. Orale: the point where the line connecting the most posterior points of I^1 alveoli meets the mesion

U. The middle point along the posterior part of the alveolus of M^1
V. The most posterior point of the palate on the mesion
W. The most ventral point in the mesion between occipital condyles

Definition for measurements (Barnett et al., 2008)

1. The distance from R to the most posterior point of the upper canine alveolus.
2. The shortest distance between the most dorsal point of the malar - temporal suture and the most ventral edge of the zygomatic arch.
3. The distance between the tip of the postorbital process of malar and the point where lacrimal - frontal suture crosses the edge of orbit.
4. The longest distance between the tip of postorbital process of the frontal and the edge of the orbit (if the tip of postorbital process of the frontal is not pointy enough, use the middle point of the rounded tip).
5. The distance along the buccal surface of an upper canine between the unworn tip and the point on the cemento - enamel junction where the medio-lateral breadth of the canine becomes its maximum.
6. The distance between the most anterior point of the buccal part of Pm^4 and the most posterior point of the tooth.
7. The greatest distance between W and the sagittal crest around its posterior end. In most case, it is easy to measure this distance. However, if the sagittal crest of the skull is very well developed, measure the distance between W and the sagittal crest around the middle point between H" and B.
8. The distance along the buccal surface of a lower canine between the unworn tip and the point on the cemento - enamel junction where the medio-lateral breadth of the canine becomes its maximum.
9. The distance between most anterior and posterior points of M1.
10. The distance between the most anterior point of canine alveolus and the most posterior point of M^1 alveolus.
11. The smallest of the greatest diameter of the section which cuts the mandible in front of the most anterior point of Pm^3 alveolus: usually an oblique measurement like the one shown in *Figure S1*.
12. The smallest of the greatest diameter of the section which cuts the mandible just behind the most posterior point of M^1 alveolus: usually a vertical or slightly oblique measurement shown in the *Figure S1*.
13. The greatest distance between P and the dorsal part of coronoid process.
14. The greatest distance between P and the dorsal part of the condyle just outside the place where the superior notch crosses the condyle: usually an oblique measurement shown in *Figure S1*.
15. The greatest distance between the ventral part of condyle just inside the place where the condyle meets the inferior notch and the dorsal part of the coronoid process: usually an oblique measurement shown in *Figure S1* – in most cases use the point on the coronoid process that was used for measurement 13.
16. Maximum width of the mandibular condyle.
17. The greatest breadth of the rostrum just above the canine alveoli.
18. The breadth of the nasal aperture above the most outer points of the I^3 alveoli.
19. The smallest distance between the infraorbital foramina.
20. Interorbital breadth: the smallest distance between the orbits.

21. The distance between the most outer points of the postorbital process of the frontal.
22. Postorbital breadth: the smallest breadth of the postorbital constriction.
23. Mastoid breadth: the distance between the most outer points of the mastoidal processes.
24. The distance between zygions: the most outer points of the mastoidal processes.
25. The greatest antero-posterior length of an upper canine at the cemento-enamel junction: the smaller diameter of the canine.
26. The greatest medio-lateral length of an upper canine at the cemento-enamel junction: the smaller diameter of the canine.
27. Pm^4-I: between the inner process and the most anterior outer process, and Pm^4-II: between the former and the second most anterior outer process of the tooth.
28. The smallest distance between the M^1 alveoli.
29. The greatest distance between the Pm^4 alveoli.
30. The distance between the most anterior/inner meeting point between the tympanic bulla and the external auditory meatus and the most anterior meeting point between the tympanic bulla and the foramen lacerum posterius.
31. The greatest length of the tympanic bulla excluding styloid process and other processes attached to the tympanic bulla: fix one end of the calliper at the point where the foramen lacerum medius meets the most prominent styloid process, and measure the greatest distance between that point and the posterior part of the tympanic bulla.
32. The greatest mediolateral distance of the vault of the tympanic bulla: the smaller diameter of the vault of the tympanic bulla.
33. The greatest breadth of the occipital condyles.

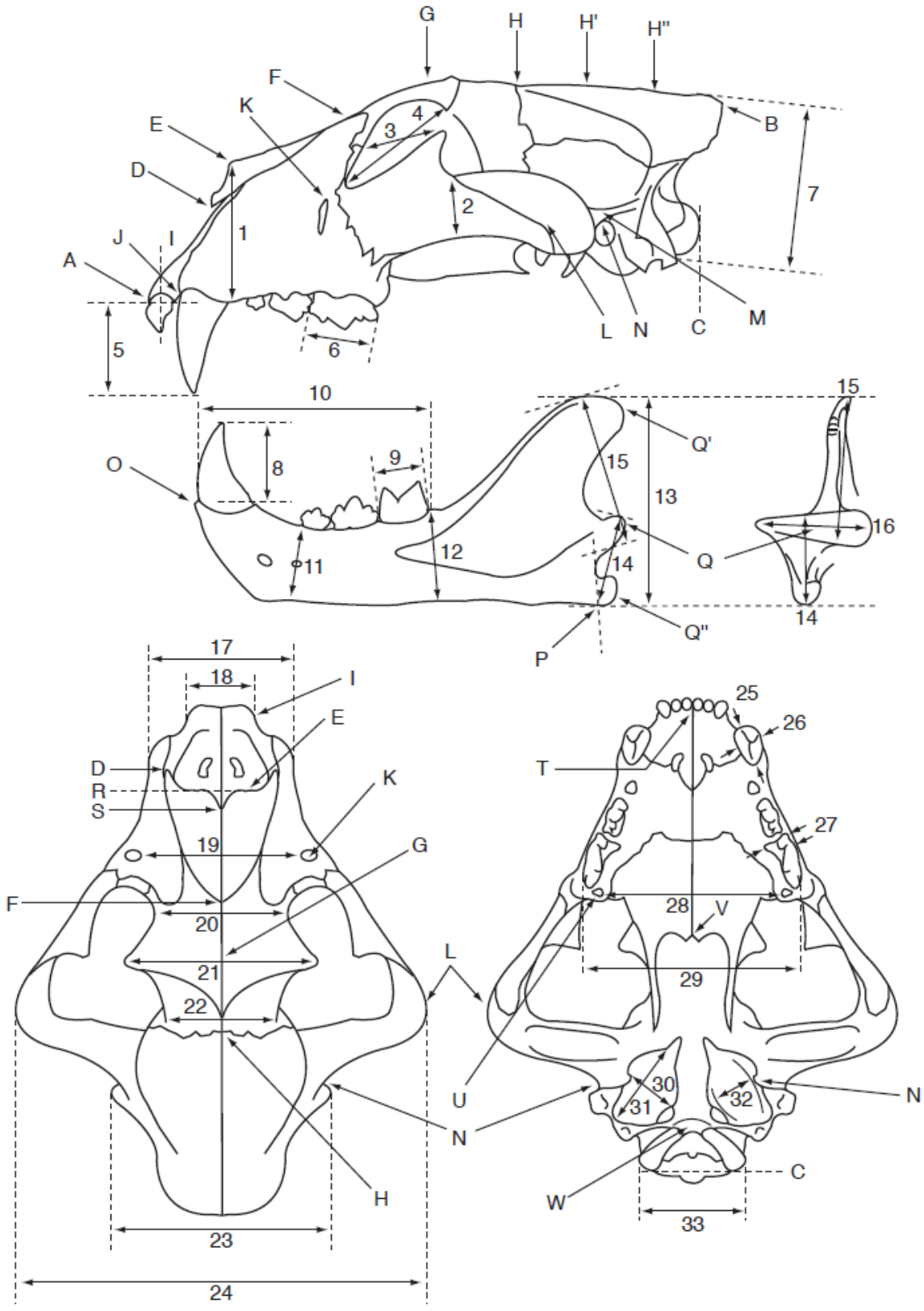


Figure S1: Figure from (Barnett et al., 2008) which corresponds to the preceding *Table S1* of measurements, **Definition of points**, and **Definition of measurements** sections.

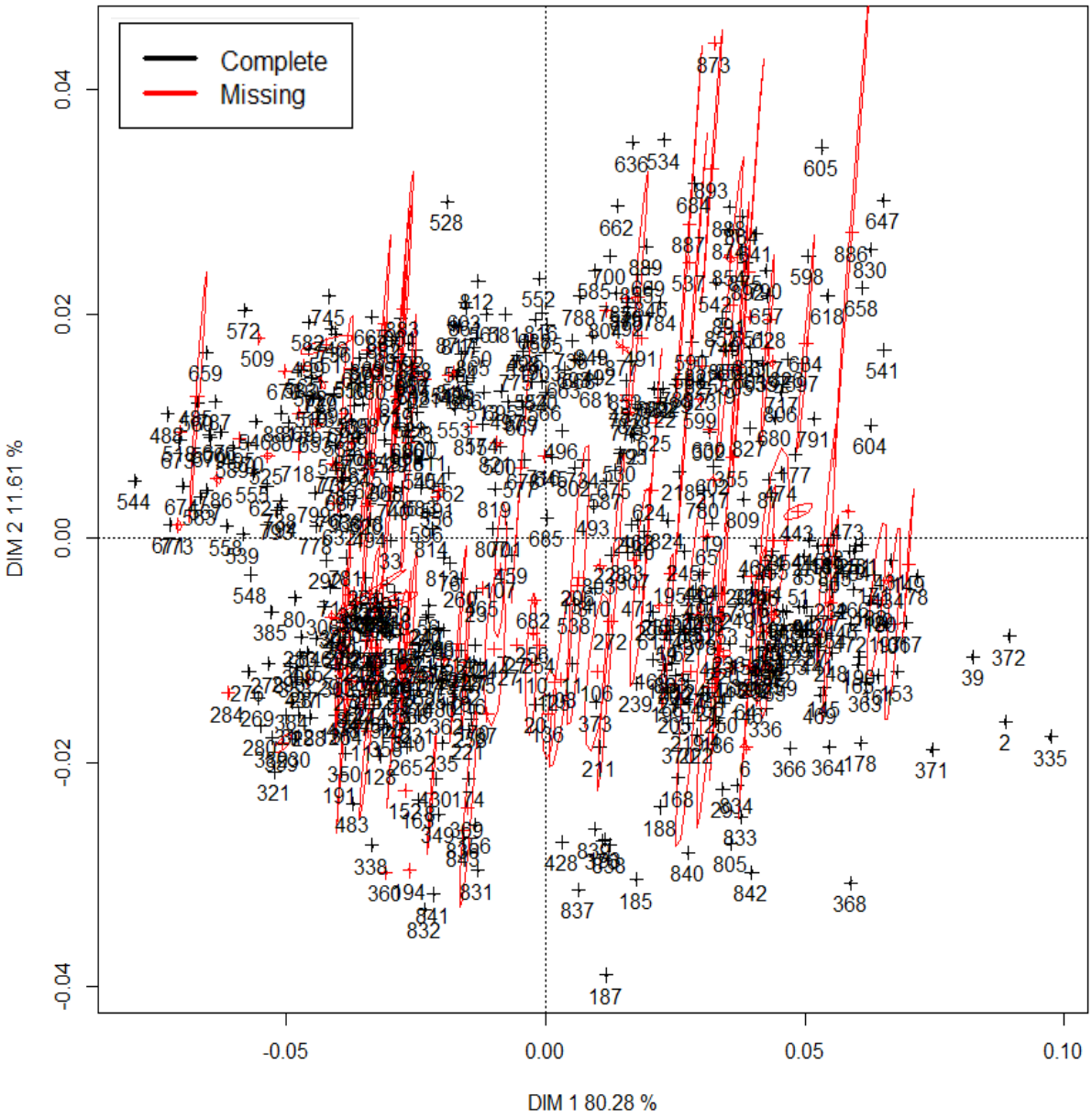


Figure S2: The effect of multiple imputation of chained equations (MICE) on a Principal Component Analysis of the imputed data. The figure displays 95% confidence ellipses associated with each imputed specimen. Specimens with no missing data are shown in black, without confidence ellipses.

Table S2: t-test p-values between captive and wild samples of scaled variables, when analysed together (All data), for female and male lions (FL, ML), female and male tigers (FT,MT), female and male *P.t.altaica* (FTA + MTA), female and male *P.t. sumatrae* (FTS, MTS) and female and male *P.t.leo* (FLL, MLL). p-values below 0.0009 Bonferroni correction are highlighted in red.

	All Data	FL	ML	FT	MT	FTA	MTA	FTS	MTS	FLL	MLL
Cranial volume (ml)	0.07	0.00	0.00	0.00	0.01	0.00	0.09	0.66	0.03	0.08	0.03
Frontal breadth	0.00	0.16	0.01	0.00	0.00	0.00	0.00	0.03	0.78	0.04	0.40
Greatest length	0.76	0.20	0.70	0.74	0.12	0.75	0.17	0.42	0.86	0.68	0.50
Condylobasal length	0.01	0.01	0.04	0.96	0.51	0.54	0.67	0.74	0.03	0.10	0.08
Palate-inion	0.38	0.98	0.11	0.67	0.81	0.99	0.64	0.68	0.38	0.42	0.14
Nasal-inion	0.76	0.10	0.01	0.28	0.00	0.35	0.92	0.09	0.50	0.12	0.00
Facial length	0.09	0.23	0.47	0.96	0.77	0.01	0.03	0.47	0.16	0.93	0.09
Head length	0.90	0.16	0.00	0.00	0.00	0.04	0.00	0.01	0.20	0.02	0.02
Bizygomatic breadth	0.00	0.00	0.00	0.00	0.05	0.01	0.35	0.03	0.34	0.00	0.00
Zygomatic length	0.01	0.05	0.57	0.74	0.33	0.81	0.83	0.30	0.00	0.46	0.69
Zygomatic length anterior	0.00	0.01	0.00	0.20	0.37	0.20	0.23	0.01	0.09	0.14	0.08
Zygomatic length posterior	0.00	0.00	0.00	0.00	0.89	0.27	0.51	0.40	0.65	0.00	0.00
Orbit vertical	0.06	0.98	0.00	0.91	0.81	0.05	0.34	0.29	0.09	0.70	0.00
Orbit horizontal	0.00	0.09	0.40	0.23	0.43	0.17	0.59	0.53	0.60	0.11	0.49
Postorbital bar	0.42	0.19	0.87	0.79	0.42	0.22	0.92	0.10	0.19	0.08	0.18
Facial length anterior	0.37	0.86	0.04	0.00	0.01	0.00	0.01	0.00	0.03	0.75	0.17
Facial length posterior	0.00	0.02	0.04	0.02	0.00	0.39	0.81	0.33	0.90	0.66	0.03
Sagittal crest	0.00	0.02	0.04	0.50	0.71	0.18	0.00	0.12	0.73	0.34	0.27
Cranial height-I	0.73	0.00	0.15	0.39	0.03	0.19	0.00	0.26	0.53	0.19	0.89
Cranial height-II	0.16	0.03	0.05	0.08	0.76	0.63	0.01	0.50	0.18	0.05	0.94
Cranial height-III	0.01	0.29	0.14	0.02	0.70	0.62	0.00	0.52	0.88	0.69	0.45
Cranial height -IV	0.01	0.08	0.07	0.15	0.00	0.00	0.00	0.24	0.13	0.19	0.04
Interorbital breadth	0.00	0.00	0.03	0.33	0.05	0.00	0.00	0.19	0.25	0.00	0.10
Postorbital breadth	0.00	0.01	0.00	0.16	0.19	0.28	0.01	0.02	0.01	0.86	0.02
Nasal length-I	0.02	0.22	0.00	0.00	0.00	0.37	0.02	0.22	0.66	0.56	0.08
Nasal breadth	0.09	0.06	0.06	0.00	0.05	0.12	0.62	0.26	0.82	0.07	0.29
Breadth between infra orbital foramina	0.00	0.00	0.00	0.61	0.00	0.01	0.00	0.03	0.35	0.03	0.19
Rostral depth-I	0.00	0.22	0.67	0.00	0.01	0.00	0.02	0.01	0.00	0.35	0.53
Rostral depth-II	0.00	0.10	0.14	0.00	0.00	0.00	0.00	0.04	0.00	0.36	0.21
Rostral breadth	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.94	0.00	0.04	0.03
Nasal aperture	0.00	0.11	0.00	0.91	0.01	0.00	0.00	0.57	0.08	0.28	0.01
Upper jaw	0.46	0.41	0.03	0.82	0.00	0.46	0.02	0.24	0.41	0.83	0.05
Palate length	0.00	0.02	0.00	0.71	0.53	0.51	0.42	0.84	0.27	0.14	0.00
Palate breadth-I	0.00	0.00	0.00	0.42	0.10	0.01	0.21	0.62	0.98	0.03	0.00
Palate breadth-II	0.00	0.00	0.00	0.00	0.00	0.55	0.61	0.38	0.13	0.00	0.00
Canine - Pm^4 (alveolus - alveolus)	0.20	0.70	0.60	0.90	0.00	0.23	0.20	0.08	0.83	0.27	0.17
Pm^2 - Pm^4 (alveolus - alveolus)	0.00	0.06	0.00	0.64	0.00	0.16	0.05	0.02	0.06	0.68	0.82
Pm^4 length	0.00	0.00	0.00	0.00	0.02	0.00	0.77	0.03	0.08	0.28	0.31
Mastoid breadth	0.00	0.00	0.00	0.26	0.00	0.07	0.05	0.10	0.10	0.00	0.00
Skull height-I	0.07	0.07	0.02	0.78	0.06	0.09	0.20	0.63	0.20	0.02	0.15
Skull height-II	0.18	0.18	0.45	0.05	0.97	0.31	0.01	0.50	0.23	0.04	0.76
Foramen magnum height	0.00	0.00	0.00	0.23	0.83	0.05	0.72	0.57	0.66	0.07	0.00
Occipital condyles breadth	0.00	0.01	0.00	0.01	0.16	0.00	0.03	0.00	0.03	0.07	0.00
Mandible length	0.20	0.03	0.01	0.89	0.05	0.97	0.68	0.74	0.03	0.05	0.12
Mandible length coronoid process	0.60	0.03	0.48	0.07	0.33	0.50	0.95	0.07	0.56	0.38	0.06
Mandible length angular process	0.00	0.00	0.00	0.23	0.00	0.47	0.49	0.59	0.33	0.14	0.01
Mandible height	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.57	0.04	0.04	0.01
Mandible height angular process	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.63	0.11	0.10	0.18
Mandible height coronoid process	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.53	0.42	0.05	0.01
Width of the mandibular condyle	0.00	0.00	0.00	0.40	0.08	0.65	0.43	0.03	0.36	0.07	0.00
Mandible depth-I	0.00	0.02	0.00	0.00	0.00	0.00	0.01	0.02	0.08	0.19	0.03
Mandible depth-II	0.03	0.21	0.66	0.49	0.24	0.28	0.04	0.11	0.71	0.06	0.79
Canine - M^1 (alveolus - alveolus)	0.14	0.36	0.95	0.60	0.00	0.78	0.05	0.01	0.83	0.67	0.66
Pm^3 - M^1 (alveolus - alveolus)	0.00	0.00	0.01	0.01	0.25	0.20	0.06	0.04	0.11	0.18	0.32
Pm^4 length	0.00	0.01	0.00	0.50	0.30	0.01	0.31	0.41	0.38	0.06	0.57
M^1 length	0.00	0.00	0.00	0.01	0.26	0.08	0.01	0.05	0.04	0.04	0.00

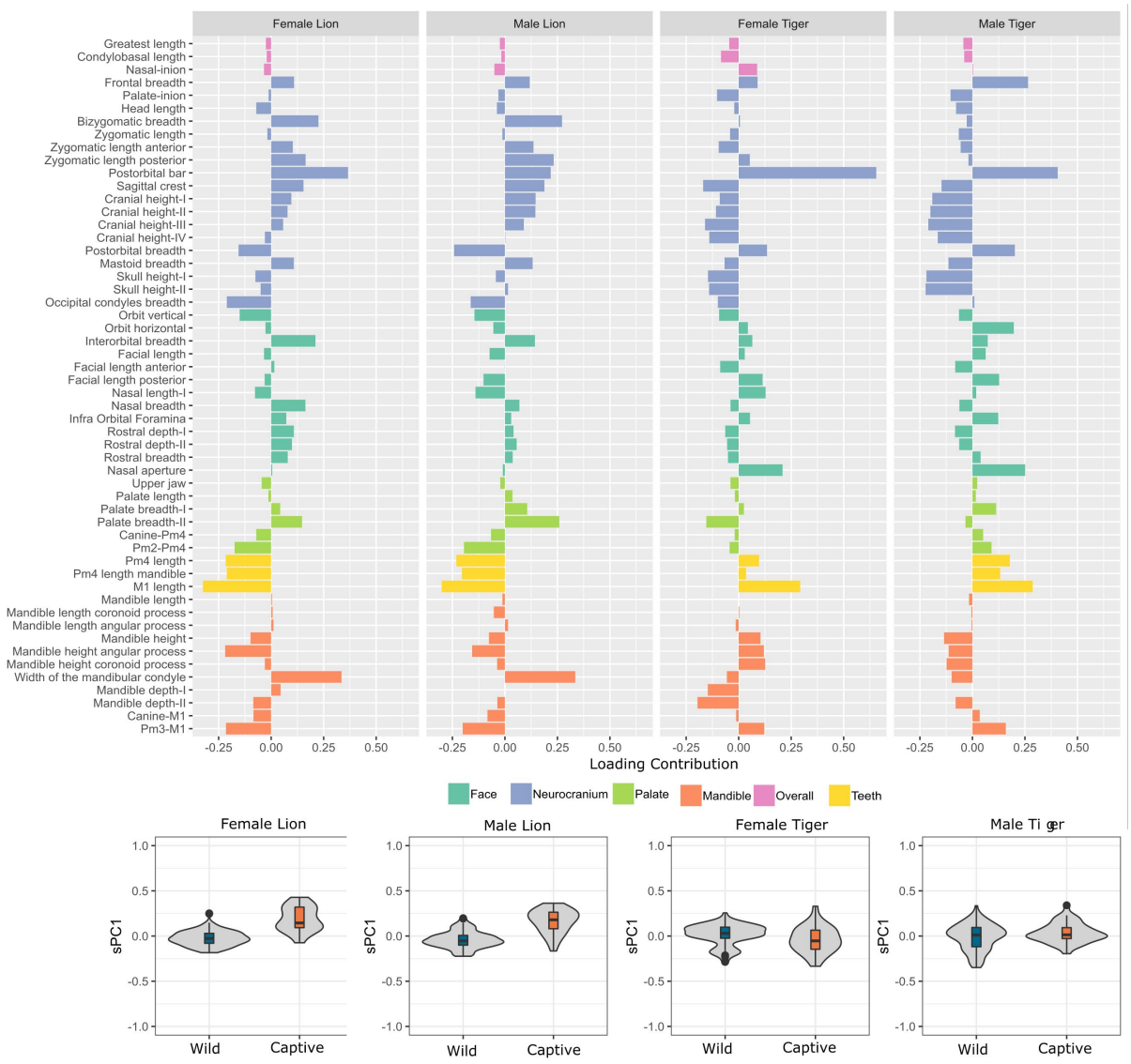


Figure 13: Loading contributions of sPC1 for male and female lions and tigers. The distributions of captive and wild specimens are shown across sPC1 using violin plots.

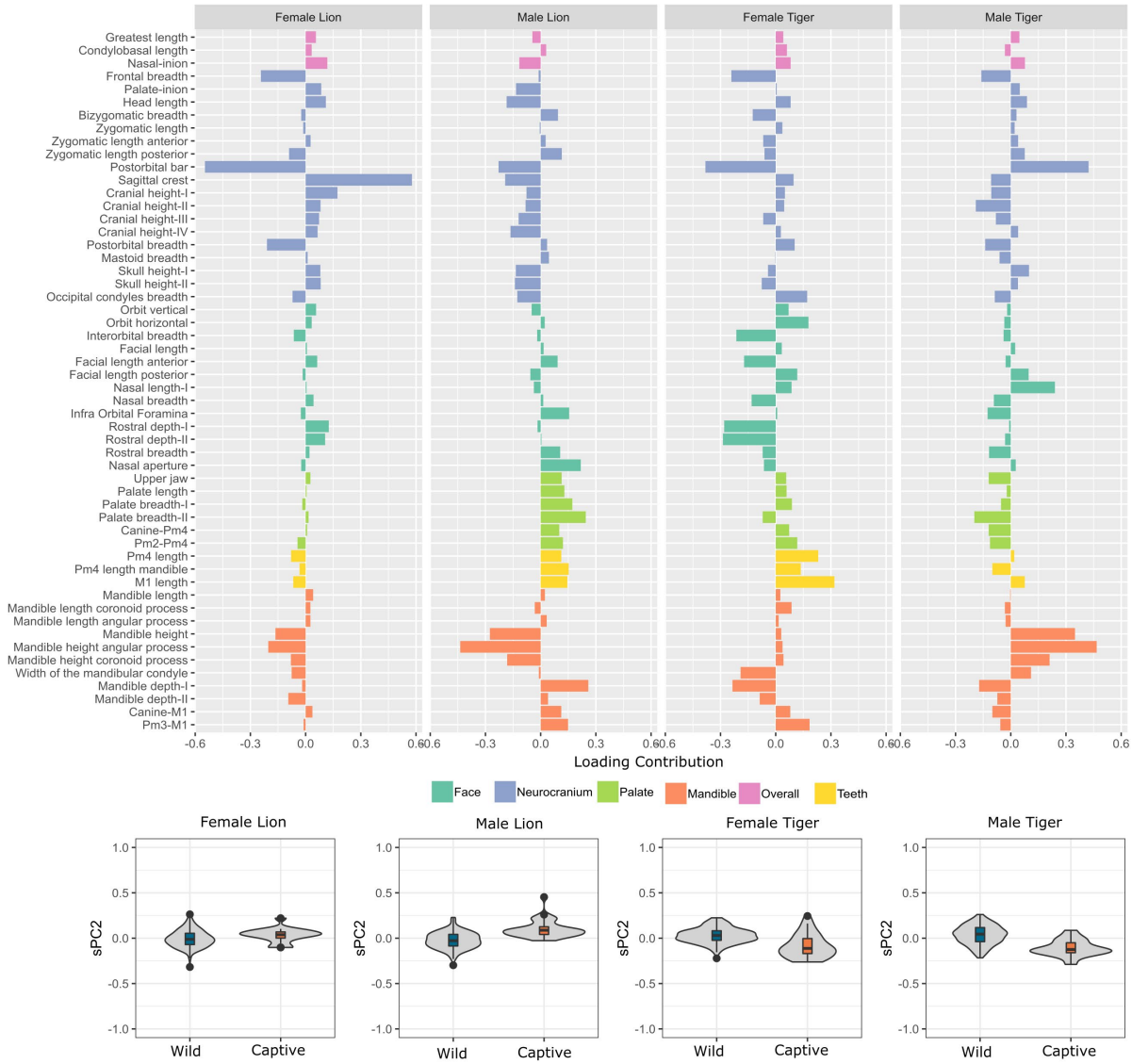


Figure 14: Loading contributions of sPC2 for male and female lions and tigers. The distributions of captive and wild specimens are shown across sPC2 using violin plots.

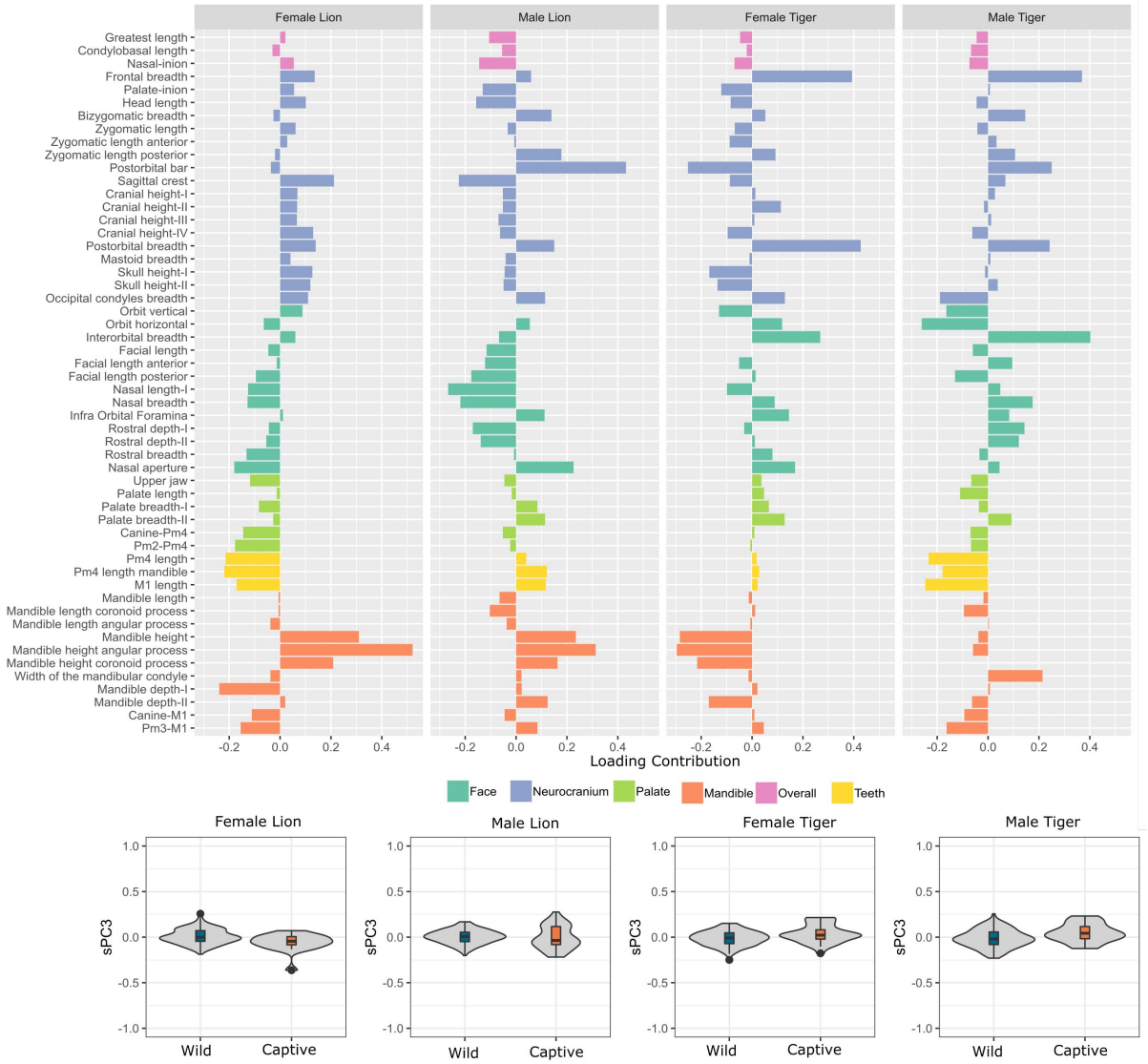


Figure 15: Loading contributions of sPC3 for male and female lions and tigers. The distributions of captive and wild specimens are shown across sPC3 using violin plots.

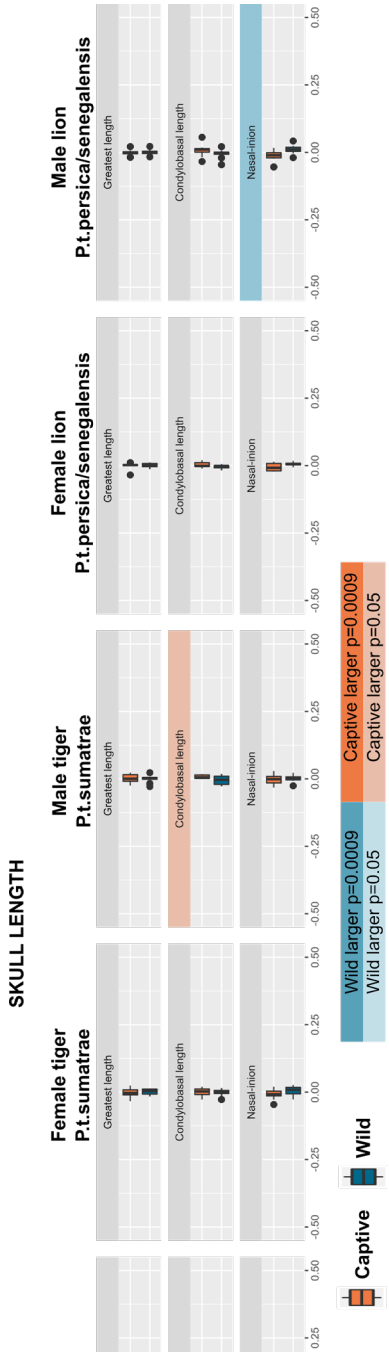


Figure S6: Variation of size independent (scaled) variables by captivity status for measurements of overall skull length when the data is split by nominal grouping. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. Significance is determined using t-tests (Table S2) based on values of 0.05 and after a Bonferroni Correction, 0.0009.

Figure S7: Variation of size independent (scaled) variables by captivity status for measurements of the rostrum when the data is split by nominal grouping. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. Significance is determined using t-tests (Table S2) based on values of 0.05 and after a Bonferroni Correction, 0.0009.

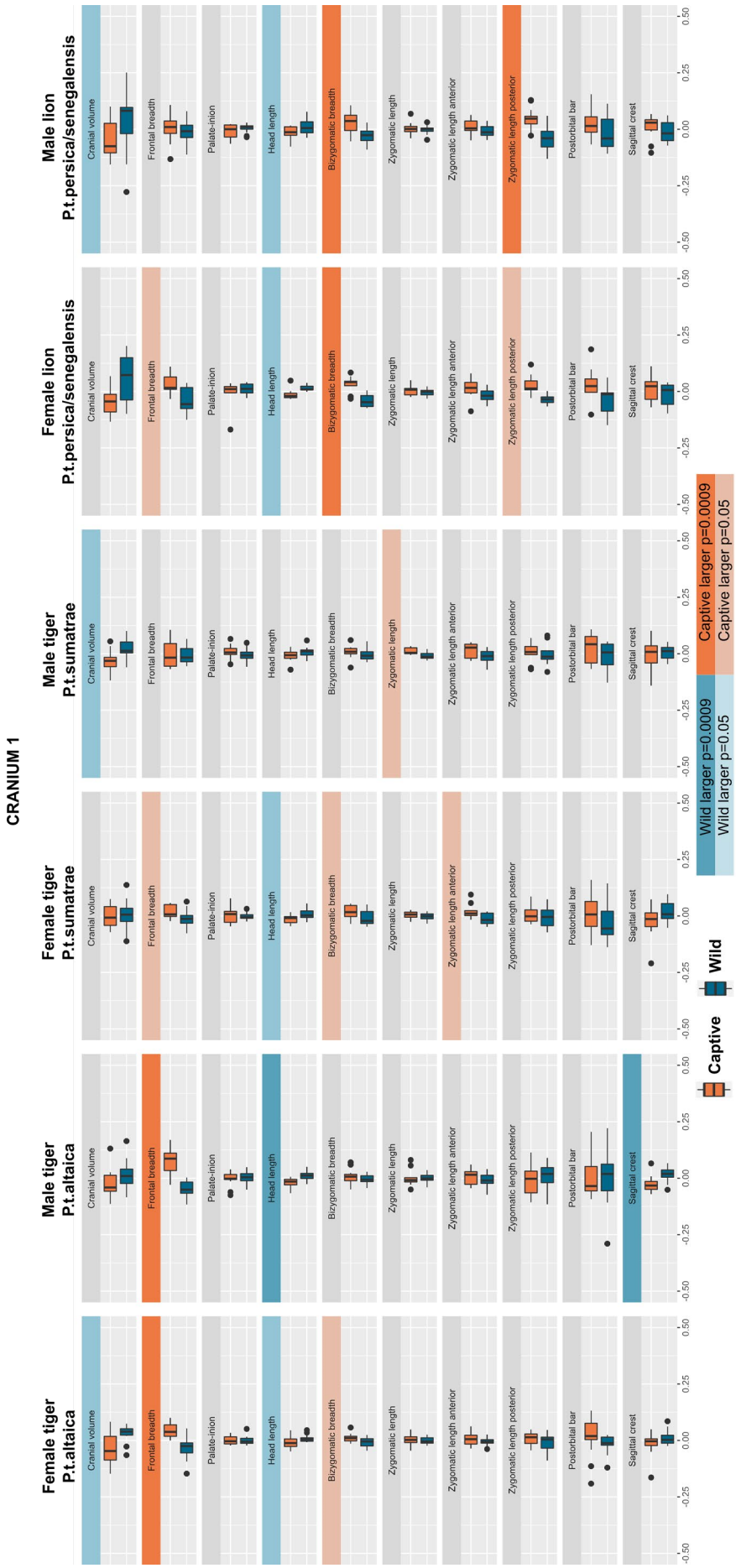


Figure S8: Variation of size independent (scaled) variables by captivity status for measurements of the rostrum when the data is split by nominal grouping. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. Significance is determined using t-tests (Table S2) based on values of 0.05 and after a Bonferroni Correction, 0.0009.

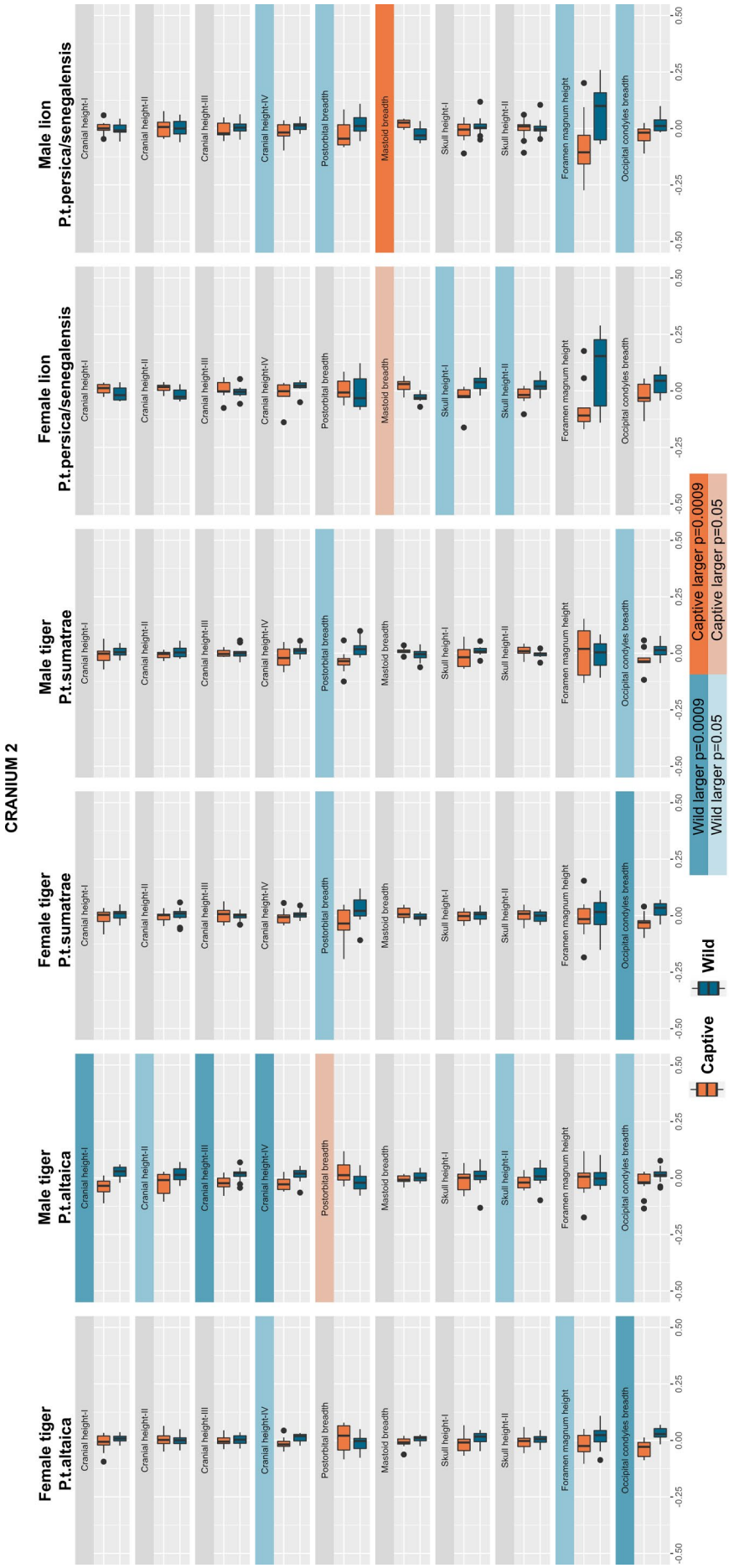


Figure S9: Variation of size independent (scaled) variables by captivity status for measurements of the rostrum when the data is split by nominal grouping. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. Significance is determined using t-tests (Table S2) based on values of 0.05 and after a Bonferroni Correction, 0.0009.

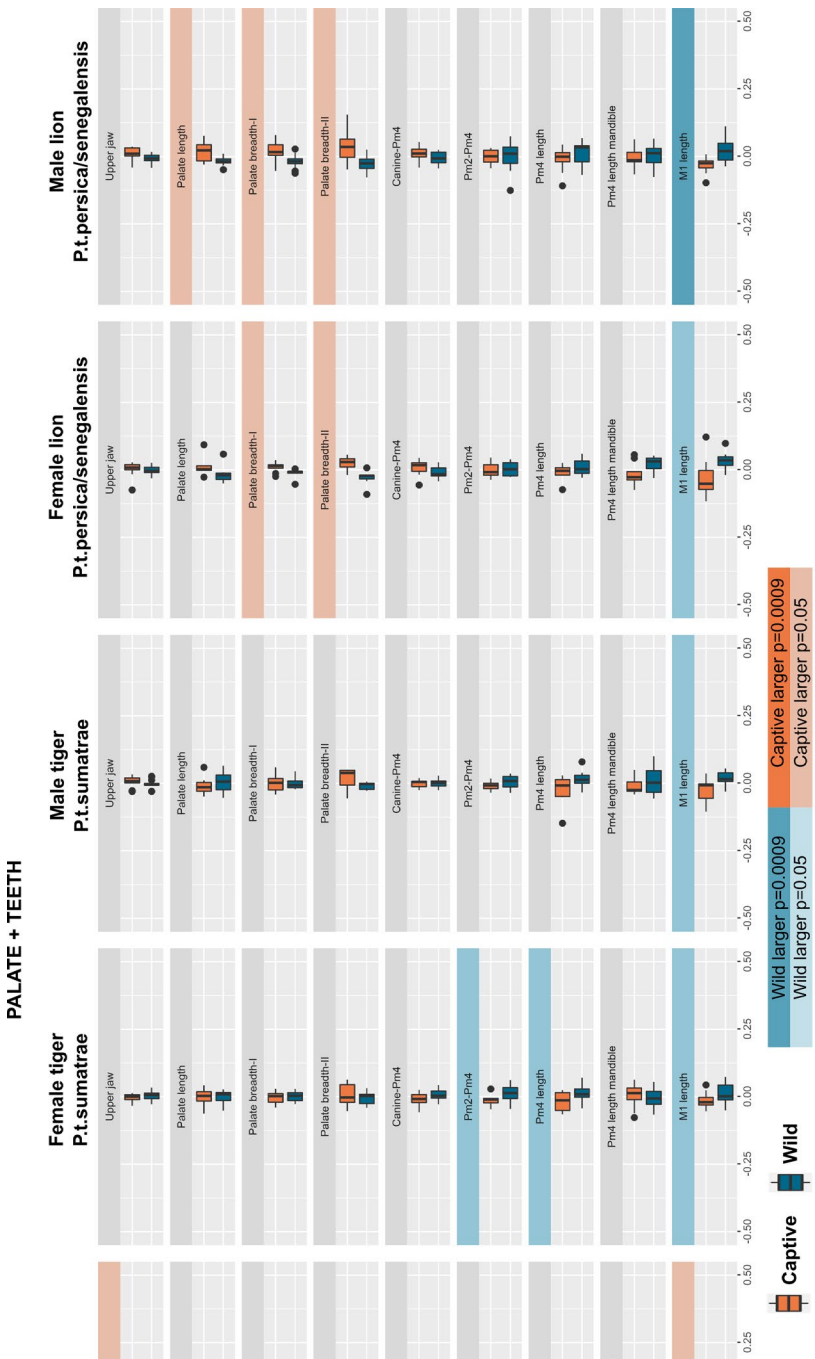


Figure S10: Variation of size independent (scaled) variables by captivity status for measurements of the palate and teeth when the data is split by nominal grouping. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. Significance is determined using t-tests (Table S2) based on values of 0.05 and after a Bonferroni Correction, 0.0009.

Figure S11: Variation of size independent (scaled) variables by captivity status for measurements of the mandible when the data is split by nominal grouping. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. Significance is determined using t-tests (Table S2) based on values of 0.05 and after a Bonferroni Correction, 0.0009.

References

Azur M.J., Stuart E.A., Frangakis C., & Leaf P.J. (2012) NIH Public Access. **20**, 40–49.

Barnett R., Yamaguchi N., Shapiro B., & Sabin R. (2008) Ancient DNA analysis indicates the first English lions originated from North Africa. *Contributions to Zoology*, **77**, 7–16.

Baur H., Kranz-Baltensperger Y., Cruaud A., Rasplus J.Y., Timokhov A. V., & Gokhman V.E. (2014) Morphometric analysis and taxonomic revision of Anisopteromalus Ruschka (Hymenoptera: Chalcidoidea: Pteromalidae) - an integrative approach. *Systematic Entomology*, **39**, 691–709.

Baur H. & Leuenberger C. (2011) Analysis of ratios in multivariate morphometry. *Systematic Biology*, **60**, 813–825.

Bennett C.L., Booth-Binczik S.D., & Steele S.R.E. (2010) Nutritional composition and digestibility by ocelots (Leopardus pardalis) of whole animals and a commercial diet. *Zoo Biology*, **29**, 753–759.

Bond J.C. & Lindburg D.G. (1990) Carcass feeding of captive cheetahs (Acinonyx jubatus): the effects of a naturalistic feeding program on oral health and psychological well-being. *Applied Animal Behaviour Science*, **26**, 373–382.

van Buuren S. (2012) *Flexible Imputation of Missing Data.* CRC Press,

Cardini A., Jansson A.-U., & Elton S. (2007) A geometric morphometric approach to the study of ecogeographical and clinal variation in vervet monkeys. *Journal of Biogeography*, **34**, 1663–1678.

Christiansen P. (2008) On the distinctiveness of the Cape lion (Panthera leo melanochaita Smith, 1842), and a possible new specimen from the Zoological Museum, Copenhagen. *Mammalian Biology*, **73**, 58–65.

Christiansen P. & Harris J.M. (2009) Craniomandibular morphology and phylogenetic affinities of Panthera atrox : implications for the evolution and paleobiology of the lion lineage. *Journal of Vertebrate Paleontology*, **29**, 934–945.

Clavel J., Merceron G., & Escarguel G. (2014) Missing data estimation in morphometrics: How much is too much? *Systematic Biology*, **63**, 203–218.

Currey J.D. (2003) The many adaptations of bone. *Journal of Biomechanics*, **36**, 1487–1495.

Duckler G.L. (1998) An unusual osteological formation in the posterior skulls of captive tigers (Panthera tigris). *Zoo Biology*, **17**, 135–142.

Hartstone-Rose A., Selvey H., Villari J.R., Atwell M., & Schmidt T. (2014) The Three-

Dimensional Morphological Effects of Captivity. *PLoS ONE*, **9**, e113437.

Hollister N. (1917) Some effects of environment and habit on captive lions. *Proceedings of the US National Museum*, **53**, 177–193.

Howell A.B. (1917) Pathologic Skulls of Captive Lions. *Journal of Mammalogy*, **6**, 163–168.

Kapoor V., Antonelli T., Parkinson J.A., & Hartstone-Rose A. (2016) Oral health correlates of captivity. *Research in Veterinary Science*, **107**, 213–219.

Kerr K.R., Morris C.L., Burke S.L., & Swanson K.S. (2013) Apparent total tract macronutrient and energy digestibility of 1- to- 3-day-old whole chicks, adult ground chicken, and extruded and canned chicken-based diets in African wildcats (Felis silvestris lybica). *Zoo Biology*, **32**, 510–517.

Khorozyan I. (2014) Morphological variation and sexual dimorphism of the common leopard (Panthera pardus) in the Middle East and their implications for species taxonomy and conservation. *Mammalian Biology - Zeitschrift für Säugetierkunde*, **79**, 398–405.

Kitchener A.C. (1999) Tiger Distribution, Phenotypic Variation and Conservation Issues. *Riding the Tiger. Tiger Conservation in Human-Dominated Landscapes* (ed. by J. Seidensticker, J. Christie, and P. Jackson), pp. 19–39. Cambridge University Press, Cambridge.

Kitchener A.C., Breitenmoser-Wuersten C., Eizirik E., Gentry A., Werdelin L., Wilting L., Wilting A., Yamaguchi N., Abramov A. V., Christiansen P., Driscoll C.A., Duckworth J.W., Johnson W., Luo S.-J., Meijaard E., O'Donoghue P., Sanderson J., Seymour K., Bruford M., Groves C., Hoffmann M., K. N., Timmons Z., & Tobe S. (2017) A revised taxonomy of the Felidae. The final report of the Cat Classification Task Force of the IUCN/SSC Cat Specialist Group. *Cat News Special Issue 11*, .

Klingenberg C.P. (2016) Size, shape, and form: concepts of allometry in geometric morphometrics. *Development Genes and Evolution*, **226**, 113–137.

Mariani F. V & Martin G.R. (2003) Deciphering skeletal patterning: clues from the limb. *Nature*, **423**, 319–325.

Mazak J.H. (2010) Geographical variation and phylogenetics of modern lions based on craniometric data. *Journal of Zoology*, **281**, 194–209.

Mazák J.H. (2010) Craniometric variation in the tiger (Panthera tigris): Implications for patterns of diversity, taxonomy and conservation. *Mammalian Biology - Zeitschrift für Säugetierkunde*, **75**, 45–68.

Mazák J.H. & Groves C.P. (2006) A taxonomic revision of the tigers (Panthera tigris) of Southeast Asia. *Mammalian Biology*, **71**, 268–287.

Mazák V.J. (1996) *Der Tiger.* Westarp Wissenschaften, Magdeburg.

McPhee M.E. (2002) Intact carcasses as enrichment for large felids: Effects on on- and off-exhibit behaviors. *Zoo Biology*, **21**, 37–47.

Moons K.G.M., Donders R.A.R.T., Stijnen T., & Harrell F.E. (2006) Using the outcome for imputation of missing predictor values was preferred. *Journal of Clinical Epidemiology*, **59**, 1092–1101.

O'Regan H.J. & Kitchener A.C. (2005) The effects of captivity on the morphology of captive, domesticated and feral mammals. *Mammal Review*, **35**, 215–230.

Saragusty J., Shavit-Meyrav A., Yamaguchi N., Nadler R., Bdolah-Abram T., Gibeon L., Hildebrandt T.B., & Shamir M.H. (2014) Comparative skull analysis suggests species-specific captivity-related malformation in lions (Panthera leo). *PloS one*, **9**, e94527.

Schafer J.L. (1997) *Analysis of Incomplete Multivariate Data.* Chapman & Hall, London.

Schafer J.L. & Graham J.W. (2002) Missing data: our view of the state of the art. *Psychological Methods*, **7**, 147–177.

Sicuro F.L. & Oliveira L.F.B. (2011) Skull morphology and functionality of extant Felidae (Mammalia: Carnivora): A phylogenetic and evolutionary perspective. *Zoological Journal of the Linnean Society*, **161**, 414–462.

Skibiel A.L., Trevino H.S., & Naugher K. (2007) Comparison of Several Types of Enrichment for Captive Felids. *Zoo Biology*, **26**, 371–381.

Smuts G.L., Anderson J.L., & Austin J.C. (1978) Age determination of the African lion (Panthera leo). *Journal of Zoology*, **185**, 115–146.

Team R.C. (2015) R: A language and environment for statistical computing. .

Thuiller W., Lavorel S., Midgley G., Lavergne S., & Rebelo T. (2004) Relating Plant Traits and Species Distributions along Bioclimatic Gradients for 88 Leucadendron Taxa. *Ecology*, **85**, 1688–1699.

Turnbull W.D. (1970) Mammalian Masticatory Apparatus. *Fieldiana: Geology*, **18**, 149–356.

White I.R., Royston P., & Wood A.M. (2011) Multiple imputation using chained equations: Issues and guidance for practice. *Statistics in Medicine*, **30**, 377–399.

Wilting A., Courtiol A., Christiansen P., Niedballa J., Scharf A.K., Orlando L., Balkenhol N., Hofer H., Kramer-Schadt S., Fickel J., & Kitchener A.C. (2015) Planning tiger recovery: Understanding intraspecific variation for effective conservation. *Science Advances*, **1**, 1–13.

Yamaguchi N., Driscoll C.A., Werdelin L., Abramov A. V, Cuisin J., Fernholm B., Hiermeier M., Hills D., Hunter L., Johansson U.S., Kascheev V., Krohmann K., Martin

T., Pavlinov I.Y., Renoud F., Tomsett L., Mije S. Van Der, Groves C., Kitchener A.C., Nijman V., & Macdonald D.W. (2013) Locating Specimens of Extinct Tiger (Panthera tigris) Subspecies: Javan Tiger (P.T. sondaica), Balinese Tiger (P.T. balica), and Caspian Tiger (P.T. virgata), Including Previously Unpublished Specimens. *The Mammal Society of Japan*, **38**, 187–198.

Zuccarelli M.D. (2004) Comparative morphometric analysis of captive vs. wild African lion (Panthera leo) skulls. *Bios*, **75**, 131–138.

Chapter 6: Determining the drivers of morphological variation in big cats.

Preface

This chapter is the second of two modelling chapters, which assesses the patterns of craniometric variation across the ranges of wild populations of the lion and the tiger. *Chapters 3+4* are used to contextualise patterns which are likely due to long term vicariance, and *Chapter 5* is used to compare wild skull variation with the known effects of phenotypic plasticity.

Introduction

This chapter aims to ascertain the mechanisms that drive variation in cranial morphology within wild lions and tigers, by considering the evolutionary history of the lion and tiger (findings from *Chapters 3 + 4*) and the known effects of phenotypic plasticity (findings from *Chapter 5*) in relation to skull measurements from animals living in the wild. Along with genetic characteristics and biogeographical ranges, morphology plays an integral role in determining subspecific taxonomy. Craniometric morphology is used in the current classification of subspecies in the lion and tiger (Mazák, 2010; Wilting et al., 2015; Kitchener et al., 2017), yet controversy still exists over the taxonomic status of subspecies within both species, from both genetic and morphological standpoints (e.g. Mazák & Groves, 2006; Liu et al., 2018). Whilst multiple studies have analysed cranial morphometric data in both the lion and the tiger (Kitchener, 1999; Mazák & Groves, 2006; Barnett et al., 2008; Mazak, 2010; Mazák, 2010; Wilting et al., 2015), there is a lack of explicit analytical consideration of the driving factors of morphological variation.

Long-term evolutionary divergence

Phenotypic variation can be the result of multiple mechanisms operating at different spatial and temporal scales (Ledevin & Millien, 2013). Whilst there is a consistent skull morphology across all felids (Sicuro & Oliveira, 2011), differences in prey size and killing techniques may dictate differing morphological traits between species of wild felid due to long-term evolutionary processes (Carbone et al., 1999; Sunquist & Sunquist, 2002). All wild felids, except the cheetah, show similarities in hunting strategy, yet differences can occur in the killing strategy of big cats (although to some extent this will depend on prey size and so is flexible within an individual): they may target the prey's skull (Sunquist & Sunquist, 2002), the spinal cord using a nape bite, or the throat or muzzle by clamping and suffocating larger prey (Sicuro & Oliveira, 2011). Through persistent selective use, these different killing bites may lead to differences in skull, mandible and dental shape (Ewer, 1998; MacDonald & Loveridge, 2010) and also different shapes of felid forelimbs, which serve a dual role in locomotion and killing prey (Gonyea, 1976). Alternatively, the functional differences between species and populations may simply arise from the genetic fixation of phenotypic variations through the natural histories of different lineages; it may not be dictated by adaptive processes relating to bite force or killing strategy (Sicuro & Oliveira, 2011); or it may be a combination of both.

At a subspecific scale, skull shape changes may also result from population bottlenecks and genetic drift, which can lead to accelerated morphological and behavioural evolution (Cardini, 2003) and thus morphological differences within a species may be the result of recent evolutionary history. This is especially important when considering isolated current and historical populations of the tiger within the Sunda Islands, and the Russian Far East, and populations of the lion in Northwest India and North Africa. Hernández-Romero et al., (2015) found shape differences between geographical units of a mammalian carnivore are likely related to geographical barriers and the large distances between populations.

Micro-evolutionary processes

Morphological evolution in mammals can occur very rapidly within small isolated populations when they are presented with altered environmental conditions over periods

ranging from a few decades to several thousand years (Millien, 2006; Pergams & Lawler, 2009). Changes in body size can occur rapidly in response to environmental change (Gardner et al., 2011; Tomassini et al., 2014), and these rapid morphological changes can occur without the genetic signal of long-term vicariance (Greenberg et al., 1998). Whilst size is highly adaptive, shape shows a considerably smaller response than size to changing environmental conditions in vervet monkeys (*Chlorocebus pygerythrus*) (Cardini et al., 2007). The situation for lions and tigers is currently unclear.

Phenotypic plasticity

The most localized mechanism for morphological variation occurs due to the phenotypic plasticity of individuals within their respective environments. This has been well documented through studies of the morphological differences between captive and wild specimens of the lion and tiger (*see Chapter 5*), yet is less well understood across wild populations, where differences may occur due to variation in prey size, type and abundance and the effects these have on mastication, killing technique, and nutrition. The phenotypic plasticity of bone may provide an ecological advantage by allowing for a wide range of sizes that a morphotype can occupy in response to local environmental conditions (Doube et al., 2009). In grizzly bears (*Ursus arctos*), meat consumption has been linked to skull parameters as indexes of nutrition during stages of development (Mowat & Heard, 2006). As cats are obligate carnivores similar patterns are probably more likely in the lion and tiger. It is likely, therefore, that the environmental conditions present during the development of an individual will affect skull size or shape though its impact on prey size, and type and abundance (Gay & Best, 1996; Cardini et al., 2007; Hernández-Romero et al., 2015).

Biogeographical patterns

Beyond random variation, e.g. though founder effects, morphological variation in wild lions and tigers may follow established geographical and environmental patterns. Bergmann's Rule (Bernhard, 1936) states that smaller body sizes are found at lower latitudes in warmer climates and larger body size in colder climates at higher latitudes. This is apparent through skull sizes across the range of the cougar (*Puma concolor*) (Gay & Best, 1996), and within

leopards (*Panthera pardus*) from the Middle East (Khorozyan, 2014). While the processes driving Bergmann's Rule are likely multifaceted, and not universal (Ashton et al., 2000; Meiri & Dayan, 2003; Gohli & Voje, 2016), diet quality may be a key mechanism in species where it is apparent; climate drives changes in plant communities and these can affect herbivores, which in turn may directly or indirectly also affect body size in detritivores and predators (Ho et al., 2010). Patterns of morphological variation have been associated with abiotic habitat variables, likely due to their effects on habitat productivity (Viguier, 2002; Cardini et al., 2007; Platz et al., 2011). Competitive exclusion can lead to body size change in species in order to occupy a separate niche from other, competing species (Gause, 1930). It is suggested that the large body size of the tiger in Java during the middle Pleistocene is a consequence of the degree of competition from other large mammalian carnivores, which may have manifested through evolutionary adaptation, or through immigration of larger mainland tigers due to Pleistocene land bridges (Kitchener & Dugmore, 2000; Mazák & Groves, 2006; Cooper et al., 2016). Beyond body size, competitive and ecological character displacement may affect body size, or specific functional skull characteristics independently of overall size (Dayan et al., 1990; Dayan & Simberloff, 1994, 2005). The island rule (Foster, 1964) dictates that large carnivores on islands may be smaller than their mainland counterparts due to limits in resource availability, known as insular dwarfism. The characteristic skull shape of Javan/Bali tigers has been interpreted as a response to insular dwarfism and associated allometric scaling caused by landscape type and available prey species (Mazák, 2010).

High variation within female leopard skulls in the Middle East may represent morphological adaptation to different prey sizes, as female skull size correlated positively with precipitation, which affects vegetation and sizes of the herbivores it supports (Khorozyan, 2014).

It may be difficult to differentiate causation from correlation when several mechanisms for morphological variation exist (O'Hara, 2005). The puma (*Puma concolor*) is smaller at lower latitudes than at higher latitudes, following Bergmann's Rule. Lower latitudes include the range of the larger jaguar (*Panthera onca*), and so competitive exclusion may also influence body size in the puma (Gay & Best, 1996), although no step-wise change is seen in body size where the puma is sympatric with the jaguar.

In this chapter the cranial morphology of the lion and tiger across their respective ranges is examined within the context of the mechanisms of morphological variation, and potential geographical trends. Both species are considered in relation to putative subspecies classification, as well as currently recognised subspecies (Kitchener et al., 2017) to assess the validity of different taxonomic units. Furthermore, wild morphology is considered in geographical and evolutionary contexts to highlight geographical and environmental trends.

Research Questions

The following analysis is structured around four research questions:

Does skull shape or size change across the geographical ranges of the lion and tiger?

Whilst previous morphometric studies have shown similarities and differences between populations of the lion and tiger, this study utilises a very large georeferenced dataset, across the geographical range of each species, and therefore spatially explicit patterns can be assessed.

Do known phylogenetic clades or subspecies boundaries account for skull variation in the lion and tiger?

The effects of recent and long-term evolutionary patterns upon the morphology of each species is assessed in relation to recent phylogenetic classes (Bertola et al., 2016; Liu et al., 2018), and subspecies definitions (Kitchener et al., 2017). Lions from the southern cape region are further separated from other East/Southern lions to assess their proposed distinctiveness (Christiansen, 2008) and Asian lions are separated from North African lions due to their broad geographic dissociation from extant populations.

Does known phenotypic plastic variation account for skull variation in the lion and tiger?

In light of the findings from *Chapter 5*, variation in wild morphology is assessed in relation to identified patterns in measurements which correspond to phenotypic plasticity. As such this chapter aims to separate the effects of recent or long-term evolutionary history from variations in life history between populations.

Can bioclimatic data account for skull variation in the lion and tiger?

Do the changing bioclimatic conditions across the range of each species account for the variation found in each species' skull morphology? Depending on the patterns observed, biogeographical patterns may relate to selective pressures and recent evolutionary history, or to life history.

Approach and Methods

The methodology outlined in *Chapter 5*, is built upon by further analysing linear cranial measurements of wild lion and tiger specimens. The dataset of wild specimens consists of 56 robust measurements from 294 lions and 208 tigers. The percentage of imputed measurements within the dataset is 3.4% which is deemed acceptable for further analysis (Clavel et al., 2014).

The nominal classification of recorded specimens is often archaic, relating to limited individual specimens or highly constrained geographic origins (Phillimore & Owens, 2006). However, current guidance suggest that only two subspecies exist for both the lion and tiger (Kitchener et al., 2017), which may obscure geographic patterns of interest. Therefore recorded classifications are re-categorised into the six proposed phylogeographic lineages for the lion (Bertola et al., 2016) and the six proposed subspecies for the tiger (Liu et al., 2018) based upon geographic origin.

I split the dataset of wild specimens into male and female lions and tigers due to the sexual dimorphism exhibited within each species (Smuts et al., 1978; O'Regan & Kitchener, 2005;

Yamaguchi et al., 2013). Isosize and shape principal components (sPC's) are calculated for each grouping based on scaled variables. Isosize and sPC's are plotted against one another to explore the variation of individuals in relation to nominal classification and putative subspecies.

I then use the textual locality descriptions associated with the origin of each individual to georeference the dataset and conduct further spatial analysis. Dealing with the numerous sources of uncertainty when digitising locations from textual descriptions is a major obstacle for spatially analysing locality data (Guo et al., 2008). The spatial extent of the locality descriptor is a major source of uncertainty (Guo et al., 2008), which is addressed using a point radius georeferencing method (Wieczorek et al., 2004). Whilst uncertainty can be assessed using more comprehensive methods, e.g. a probabilistic approach (Guo et al., 2008), the quantity of data and requirements for storage and analysis favour the simpler approach used here. Each specimen is assigned a latitude and longitude value, and a radius of uncertainty, which relates to the scale of uncertainty of the descriptor (*Table 1*). For descriptions where a name could refer to a city or a province, the province, or larger feature is chosen. Coordinate precision, misidentification of localities, and digitisation error within the locality dataset must be considered (Muñoz et al., 2011). Therefore, where a name is ambiguous and could refer to two or more points, either no locality is given, or the locality is given a wider uncertainty radius - e.g. the reference is to a town and a region – if the town cannot be identified, it will be georeferenced to the region. Where a linear feature such as a river is referenced within a region, the start and finish of the river is taken from where it enters and exits the region.

Table 1: Georeferencing techniques used for assigning point and radial uncertainty to textual locality descriptions of a lion and tiger morphometric dataset.

Description Type	Description Example	Coordinates Given	Uncertainty Radius
Offset	15km SW of x	Coordinates offset from gazetteer entry	5km
Small Settlement	x (village)	Gazetteer entry	Distance to nearest settlement
Large Settlement	x (town/city)	Gazetteer entry	50km
Area	x (region)	Centroid of polygon	From centre point of x to furthest border of region x
Span	Between x and y	Location half way between gazetteer entries for x and y	Half the distance between x and y
Natural Feature (area)	Mount x, Saltlake x	Gazatteer entry	Distance from x to

			furthest edge of feature determined by satellite imagery
Natural Feature (line)	River x	Nearest point on feature to the straight line half way point between the ends of the feature	Distance from calculated coordinate to furthest end of feature

Latitude is often used as a climatic and environmental proxy when exploring ecogeographical patterns (Cardini et al., 2007), however the availability of environmental variables allows a direct comparison with skull morphology, which may not directly correlate with latitude. Four bioclimatic variables are prepared to assess the morphological variation across geographic and environmental space – Growing degree-days on a 0°C base (Metzger et al., 2013), Temperature Seasonality (Hijmans et al., 2005), Aridity Index and Potential Evapotranspiration Seasonality (Zomer et al., 2007, 2008). These variables exhibit low correlation between each other, and thereby allow for the comparison of bioclimatic conditions with skull metrics using a relatively low number of variables (Metzger et al., 2013). A relationship between meat intake and body size in bears at a continental scale may be affected by variation in the season of terrestrial meat intake (Mowat & Heard, 2006), and so seasonal bioclimatic variables may provide insights into changing annual productivity which could relate to skull variation in the lion and tiger, especially given their status as obligate carnivores.

By assigning an uncertainty radius for each point, the uncertainty in subsequent analysis with environmental data can be accounted for. The minimum and maximum latitude and longitude is calculated for each point within R (R Core Team, 2015). I derive mean, minimum and maximum values for continuous environmental variables using the isectpolyrst function of GME tools (Beyer, 2014). Individual specimens are removed from analysis when the range of possible environmental conditions within the individual's uncertainty range is unacceptably high, and obscures biogeographic pattern. This is better than simply removing individuals with large uncertainty radii, because within environmentally homogenous regions, the location data of individuals may have a large geographic uncertainty which has limited significance given that within the potential range of the specimen, the habitat is essentially the same. Conversely the range of environmental conditions an individual could have utilised where the uncertainty radius is small, yet the landscape is climatically heterogeneous, may be

very high. Environmental variables are compared with isosize and shape principal components to identify biogeographical trends.

Results

Specimen localities and status

Following the imputation and data removal procedures of *Chapter 5*, 151 wild male lions, 143 female lions, 112 male tigers and 96 female tiger specimens are available. Of these, 150 male lions, 142 female lions, 98 male tigers and 86 female tigers are georeferenced (*Figure 1*). The data are not randomly distributed across the former range of either the lion or the tiger. The vast majority of specimens of the lion are eastern and southern African in origin, and records from North-Africa, the Near East and Asia are lacking. Similarly there is a scarcity of tiger specimens from the Caspian region, mainland China, northern Myanmar, and the northern Malay Peninsula. Georeferencing the specimens highlights a disparity between the nominal taxonomic classification of some specimens, and currently recognised clades or subspecies. This is especially apparent for lion specimens recorded as *P.l.nubica*, which span the East African suture zone between the currently recognised Northern Lion subspecies (*P.l.leo*) and southern subspecies (*P.l.melanochaita*). Therefore lion specimens are reclassified into recognised clades (Bertola et al., 2016) and subspecies (Kitchener et al., 2017), and the tiger into proposed (Liu et al., 2018) and recognised (Kitchener et al., 2017) subspecies. Common names are used for the tiger subspecies proposed by Liu et al., (2018) to avoid confusion with the scientific names of Kitchener et al., (2017). Certain tiger specimens were reclassified due to their geographical location, for example, a recorded 'Caspian' tiger was re-classed as Bengal given its position on the Afghan/Pakistan border (see *Chapter 3*). Those specimens found within the East African suture zone between Central and North Eastern clades are re-categorised based upon which exclusive clade they are closest to (*Figure 1*).

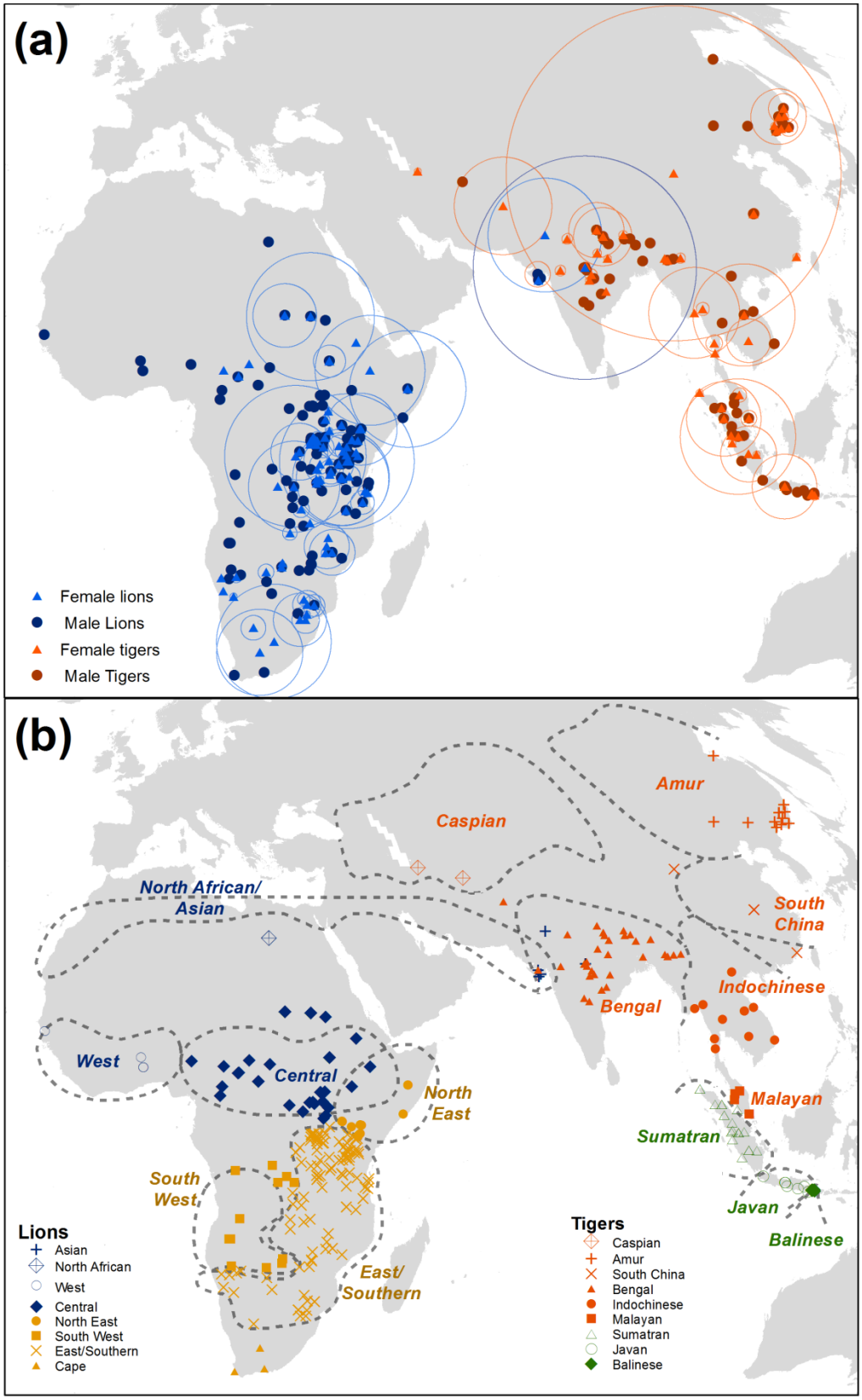


Figure 1: Distribution of georeferenced skulls of male and female lions and tigers, including the geographic uncertainty of each recorded point (**a**). Lion clades (Bertola et al., 2016) and putative tiger subspecies (Liu et al., 2018) are indicated by shape (**b**). Specimens are coloured by currently recognised subspecies (Kitchener et al., 2017).

Principal component analysis

Results for the analysis of isosize and shape principal components for male and female lions are shown in *Figure 2*. There is no visual relationship between isosize or any sPCs and therefore there is little evidence that allometric scaling affects skull shape in the lion. Isosize does not differentiate between recognised subspecies well. However, the largest female lions are exclusively *P.l.melanochaita*, and West and Asian lion clades are consistently smaller. sPC1 contributes 16.2% and 16.8% to the overall shape variance in male and female lions respectively. The low contribution of sPC1 is likely due to the removal of the influence of size upon shape. Whilst there is no apparent separation between the two recognised subspecies by the first principal component, Asian lions are separated from other lions on this axis. sPC2 and sPC3 do not differentiate between either recognised subspecies or putative classifications.

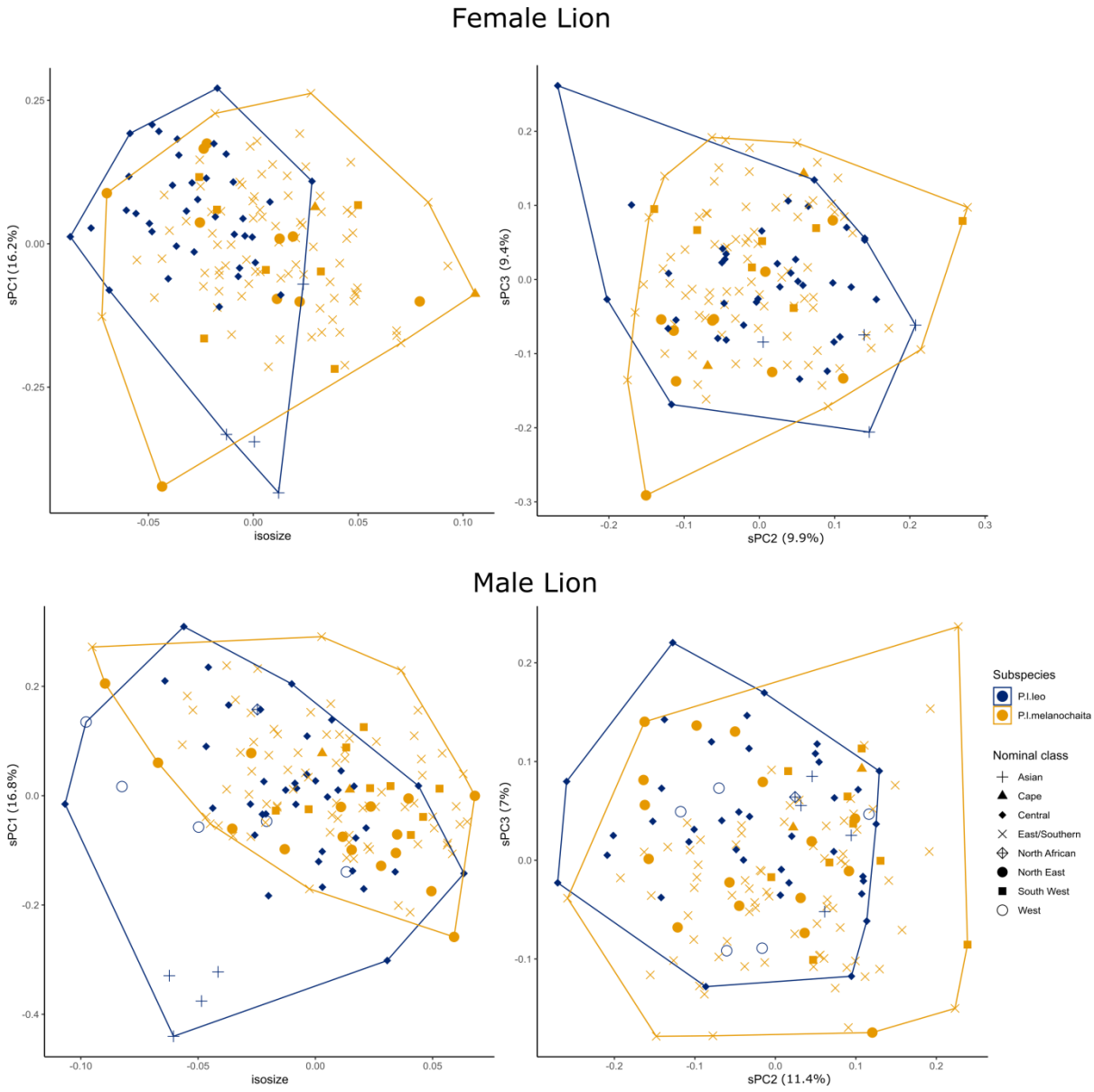


Figure 2: Isosize and shape principal components for female and male lions. Convex hulls differentiate by recognised subspecies, whilst shape represents taxonomic clades.

Isosize and sPCs are plotted for male and female tigers in *Figure 3*. Larger specimens tend to be from Continental tigers (*P.t.tigris*), although there is greater overlap in size between subspecies in female tigers. Whilst the smallest female tigers are all Sunda Island tigers (*P.t.sondaica*), Javan and Sumatran tigers can be as large as Continental tigers. Unlike lions, there is apparent allometric scaling as shown by the relationship between isosize and sPC1 in both male and female tigers. sPC1 accounts for 17.8% and 21.7% of the shape variation in female and male tigers respectively, and whilst most nominal groupings show a clinal relationship between isosize and sPC1, Amur tigers are separated by the similarly sized

Bengal and Indochinese tigers by sPC1. sPC2 and sPC3 do not separate out by recognised subspecies well, but there is strong separation of Sunda Island tigers by island of origin, especially by sPC3 in female tigers. Amur tigers are separated from the other Continental tigers by sPC2, but in male tigers they occupy similar component space to Sunda tigers. There is no clear separation of South China, Indochinese, Malayan or Bengal tigers in either sPC2 or sPC3 in male or female tigers.

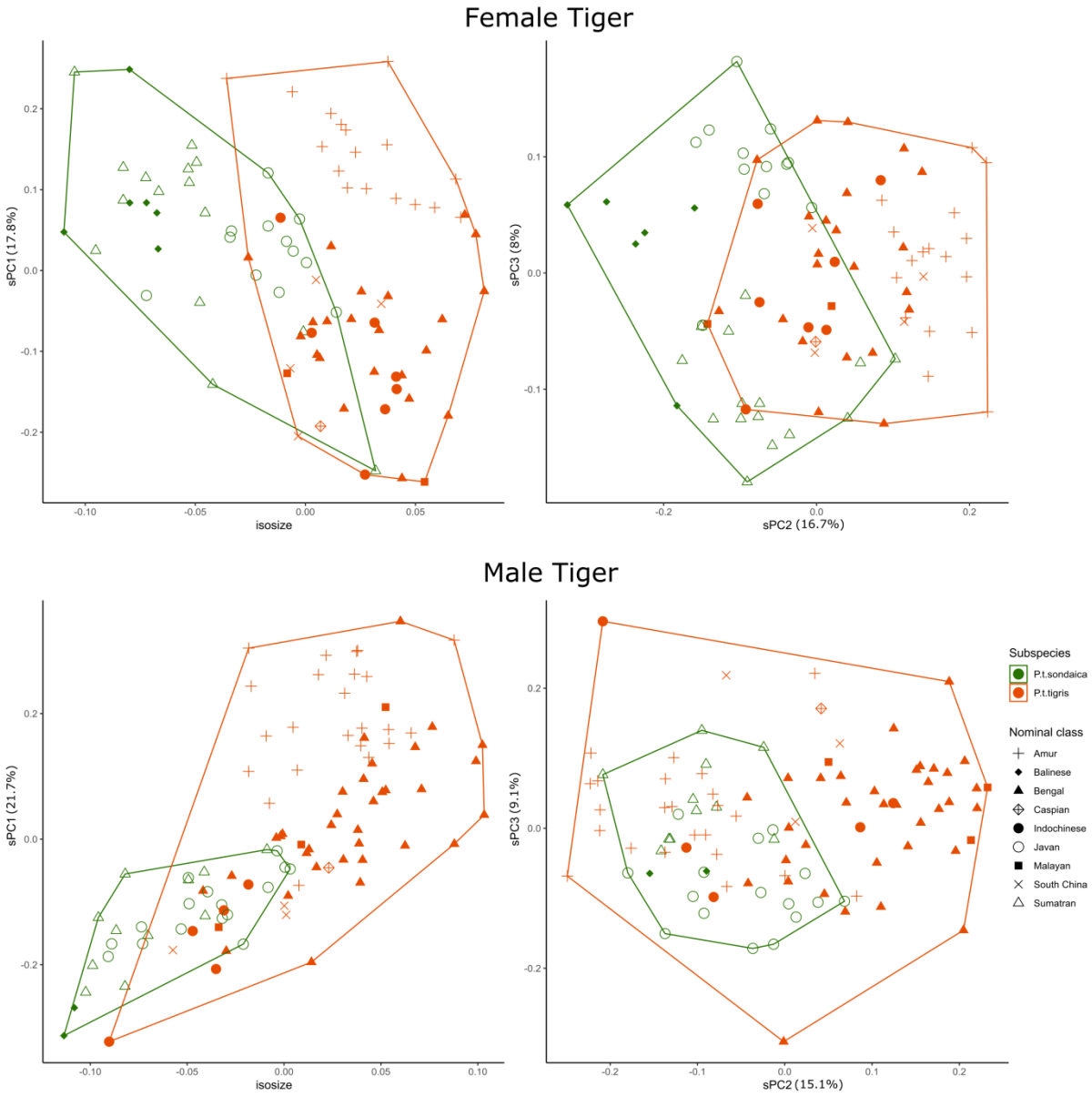


Figure 3: Isosize and shape principal components for female and male tigers. Convex hulls differentiate by recognised subspecies, whilst shape represents taxonomic clades.

The contributions of variables to sPC1 for male and female lions and tigers are shown in *Figure 4*. Cranial volume and foramen magnum height strongly contribute to sPC1 in both female and male lions, which separates Asian lions from other populations. The tail below *P.l.leo* violin plots (*figure 4*) represents the separation of Asian lions from other populations in this component. The postorbital bar and foramen magnum height contribute strongly to sPC1 in male and female tigers. Cranial and skull height measurements stand out as contributing to sPC1 in male tigers, but not in female tigers. Mandible height measurements stand out for female tigers but not for male tigers.

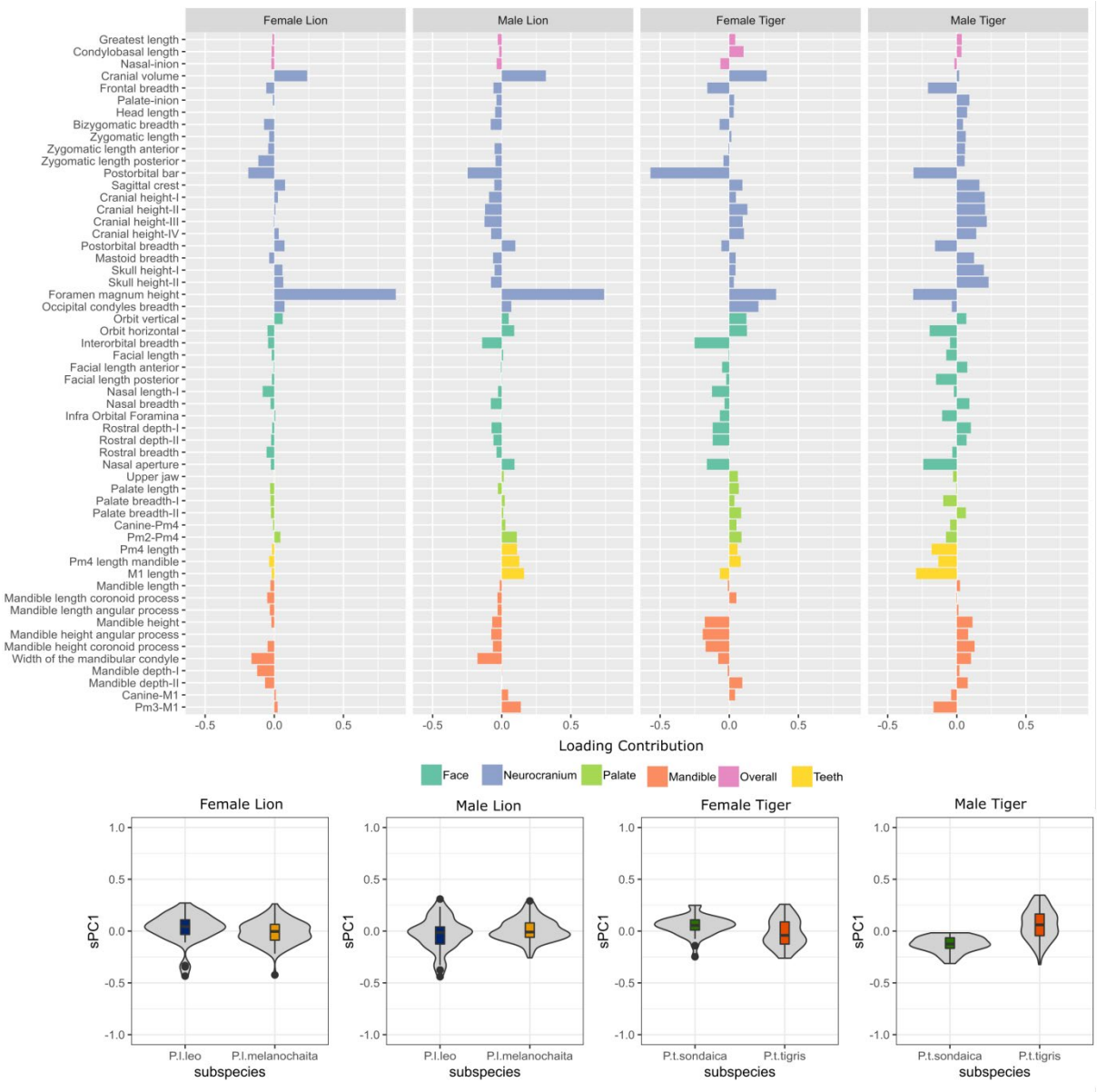


Figure 4: Loading contributions of sPC2 and sPC3 for wild tigers. The distributions of specimens from each recognised subspecies each sPC are shown using violin plots.

Given the discriminatory power of sPC2 and sPC3 in the tiger, the loading contributions for each component are shown in *Figure 5*. Foramen magnum height, M^1 length, and nasal aperture contribute strongly to sPC2 in female tigers, which suggests a latitudinal change in these measurements given that female tigers spread across sPC2 by nominal class (*Figure 3*). Whilst not as strong as other variables, there is a combined contribution of mandible height measurements in contributing to sPC2 in female tigers, which is also found in male tigers. Cranial volume and postorbital bar contribute strongly to sPC2 in male tigers. Frontal breadth, postorbital breadth, breadth between the infraorbital foramina, and measurements of facial length contribute to sPC3 of female tigers, which separates Balinese, Javan and Sumatran tigers from one another.

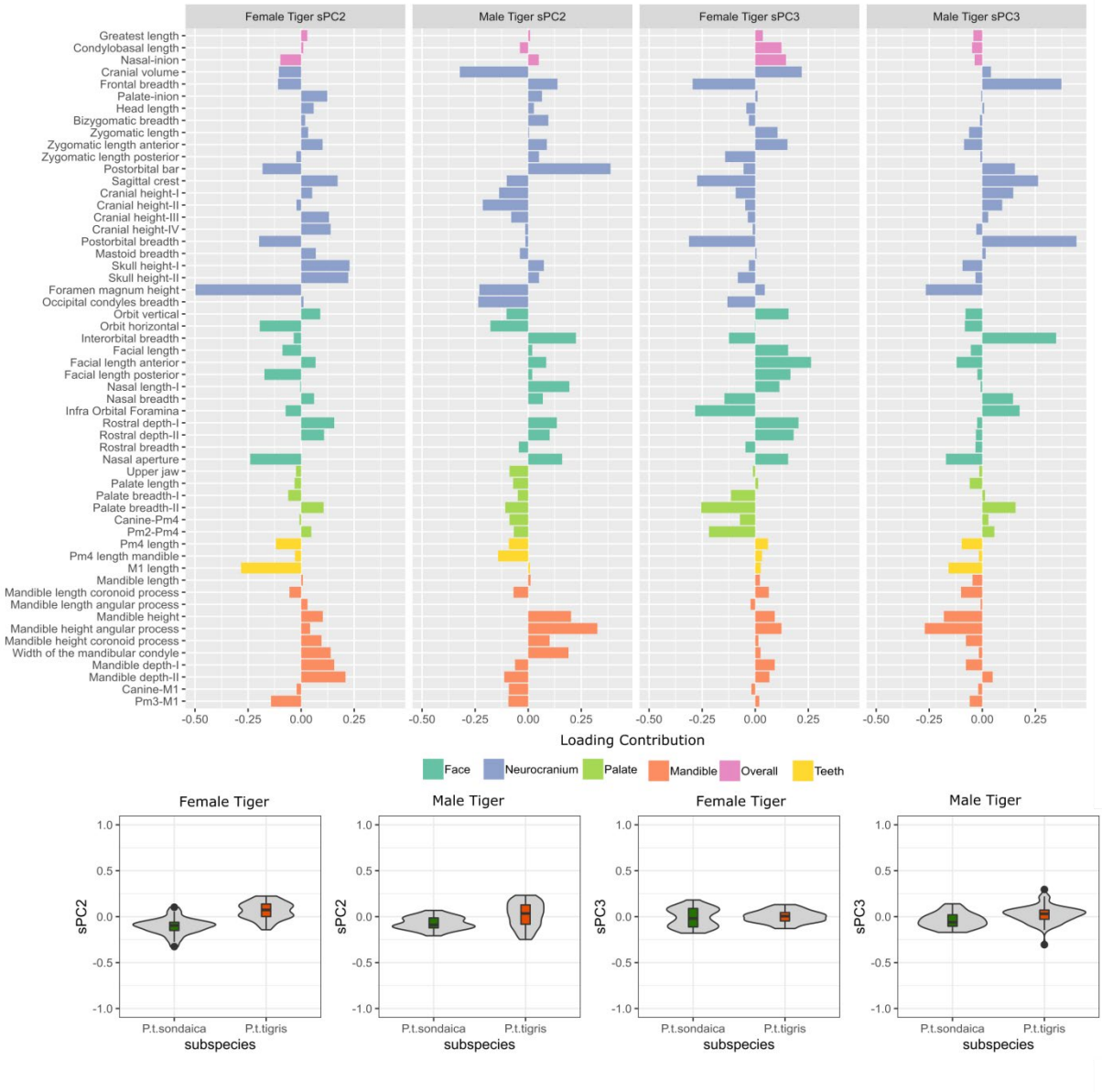


Figure 5: Loading contributions for sPC2 and sPC3 in male and female tigers. Boxplots of the distribution of sPCs are displayed by recognised subspecies.

Measurement patterns

Figures 2 + 3 highlight the differences between Asian lions and other northern lion clades, and the differences between Amur tigers and other Continental tigers. Patterns of change in measurements between the Asian lion and other northern lion clades are presented in *Figure 6, and Figures S1-S5 of the Supplementary Information*, in relation to patterns between captive and wild northern lions as outlined in *Chapter 5*. Similarly, wild Amur tigers are compared with other wild Continental tigers in relation to captive and wild differences

between Amur tigers (*Figure 7, and Figures S6-S10 of the Supplementary Information*). Wild Asian lions exhibit patterns which are more akin to captive northern lions, such as a dramatically reduced foramen magnum height and cranial volume, greater rostral and nasal breadths, and wider mandibular condyles. Whilst mandible height is not statistically different between wild Asian and other northern lions, it tends to be lower in Asian specimens. Wild Amur tigers on the other hand exhibit morphological patterns which are concordant with more 'wild' characteristics compared with other Continental tigers. Wild Amur tigers have increased cranial volume, cranial heights and longer sagittal crests, and narrower rostra and nasal width measurements when compared to other wild Continental tigers. Conversely, wild Amur tigers have reduced mandible heights and greater palate breadths which are more similar to captive specimens. The postorbital bar of wild Amur tigers is significantly smaller than other wild Continental tigers, a measurement which exhibits high variance, but no discriminatory power between captive and wild specimens (*Chapter 5*).

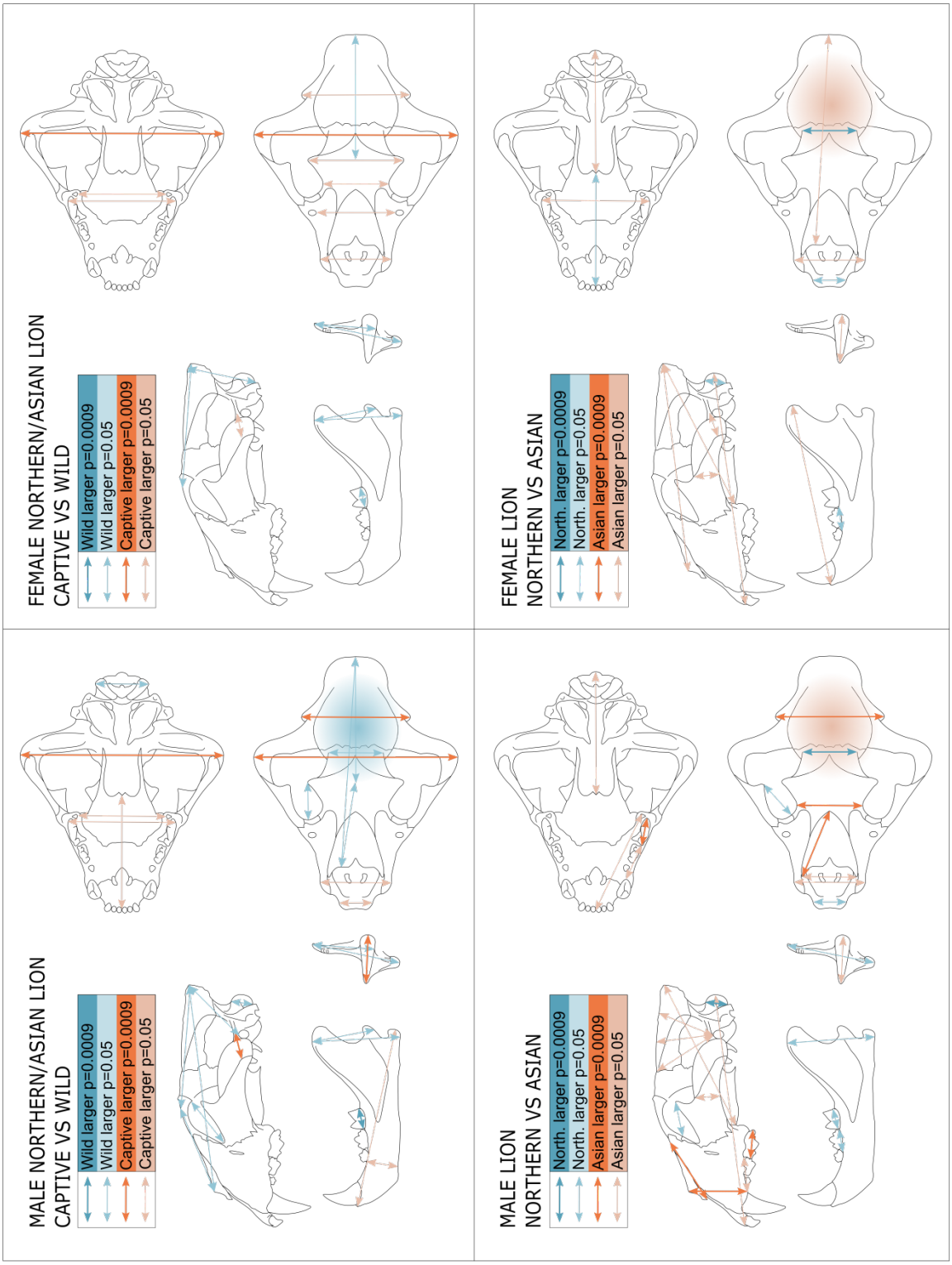


Figure 6: A graphical representation of measurements which are significantly different between different populations of the lion. Significance is determined by t-tests (*Table S1* + *Chapter 5 Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009. Differences based on captivity status are compared for both male and female lions based on the findings of *Chapter 5*. Differences between wild Asian lions and other lions of the Northern lion clade are also compared. Thereby cranial differences between wild populations can be assessed in relation to the influence phenotypic plasticity as shown by captive/wild differences.

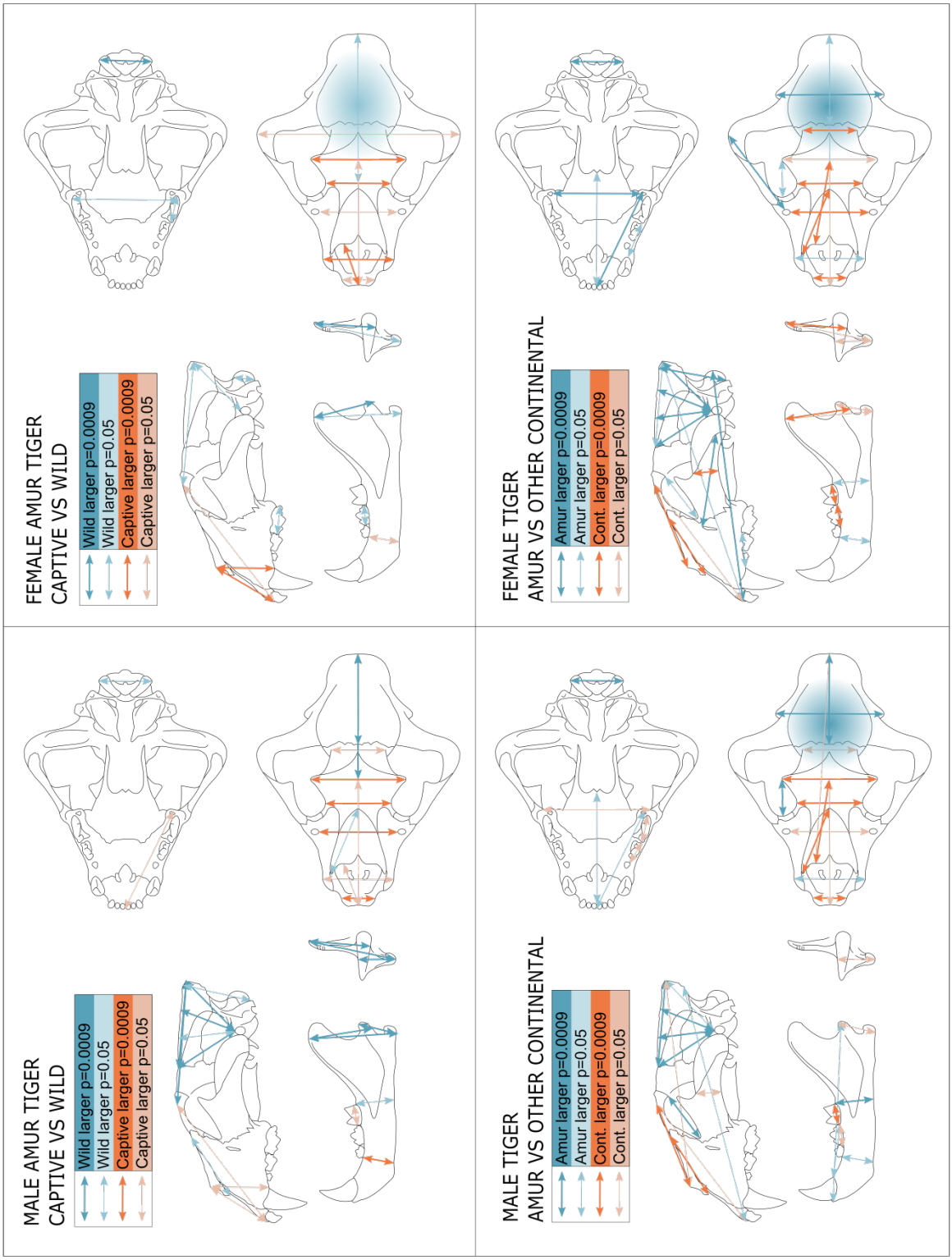


Figure 7: A graphical representation of measurements which are significantly different between different populations of the tiger. Significance is determined by t-tests (*Table S1 + Chapter 5 Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009. Differences based on captivity status are compared for both male and female tigers based on the findings of *Chapter 5*. Differences between wild Amur tigers and other Continental tigers are also compared. Thereby cranial differences between wild populations can be assessed in relation to the influence phenotypic plasticity as shown by captive/wild differences.

Patterns of change between each Sunda Island and Continental tigers (without Amur tigers) are shown in *Figures S11+S12 of the Supplementary Information* due to the separation of Sunda Island tigers in sPC2+3 (*Figure 3*). Patterns of variation between each population are not consistent with patterns found between captive and wild specimens (*Chapter 5*). For example, foramen magnum height is largest in the Balinese tiger, and smallest in Continental tigers, yet the sagittal crest is also smallest within this population. Head length in Balinese tigers is also considerably smaller than in other populations. Sumatran tigers have a smaller cranial volume than Javan tigers, but also have a noticeably greater sagittal crest length. Collectively, the Sunda Island tigers tend to have reduced mandible heights, and larger foramen magnum heights compared to Continental tigers, although more generally, patterns of change between each group of Sunda Island tigers and Continental tigers are not consistent with patterns found between captive and wild populations.

Environmental analysis

The relationship between isosize and latitude is explored for the lion and tiger (*Figure 8*) due to the clinal variation in skull size in the tiger and lion identified in *Figures 2+3*. Lion skull size decreases with decreasing latitude in both males and females, although there is considerable variation, and specimens at equatorial latitudes cover the range of skull sizes found across lions as a whole. In the tiger, skull size generally increases with latitude, although the Javan tiger is generally larger than the Sumatran tiger as expected because it is further from the equator. Step changes in latitude in the tiger represent a lack of samples surrounding the northern Malay Peninsula, and within mainland China. There are no step changes in skull size for either the lion or the tiger.

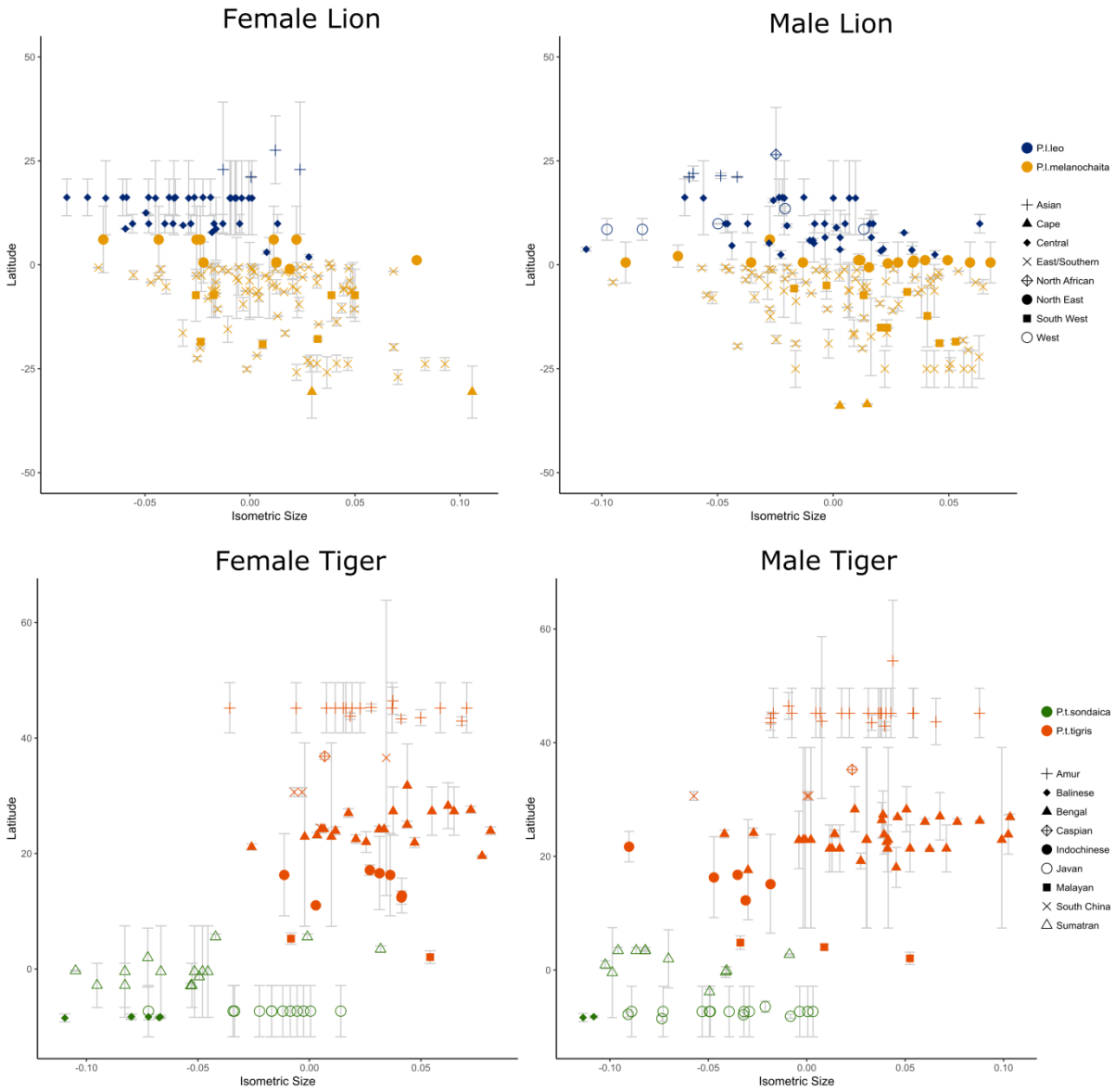


Figure 8: The relationship between latitude and isometric size in male and female lions and tigers. Error bars display the latitudinal uncertainty of each specimen given their textual locality descriptions. Specimens with very high latitudinal uncertainty have been removed.

Figures 9-12 show the relationship between skull size and four bioclimatic variables for male and female lions and tigers. Within the lion (Figures 9+10), patterns are difficult to distinguish between skull size and environment, however the results show that bioclimatic conditions for both subspecies of the lion and for different clades are relatively similar, with no apparent step changes in conditions.

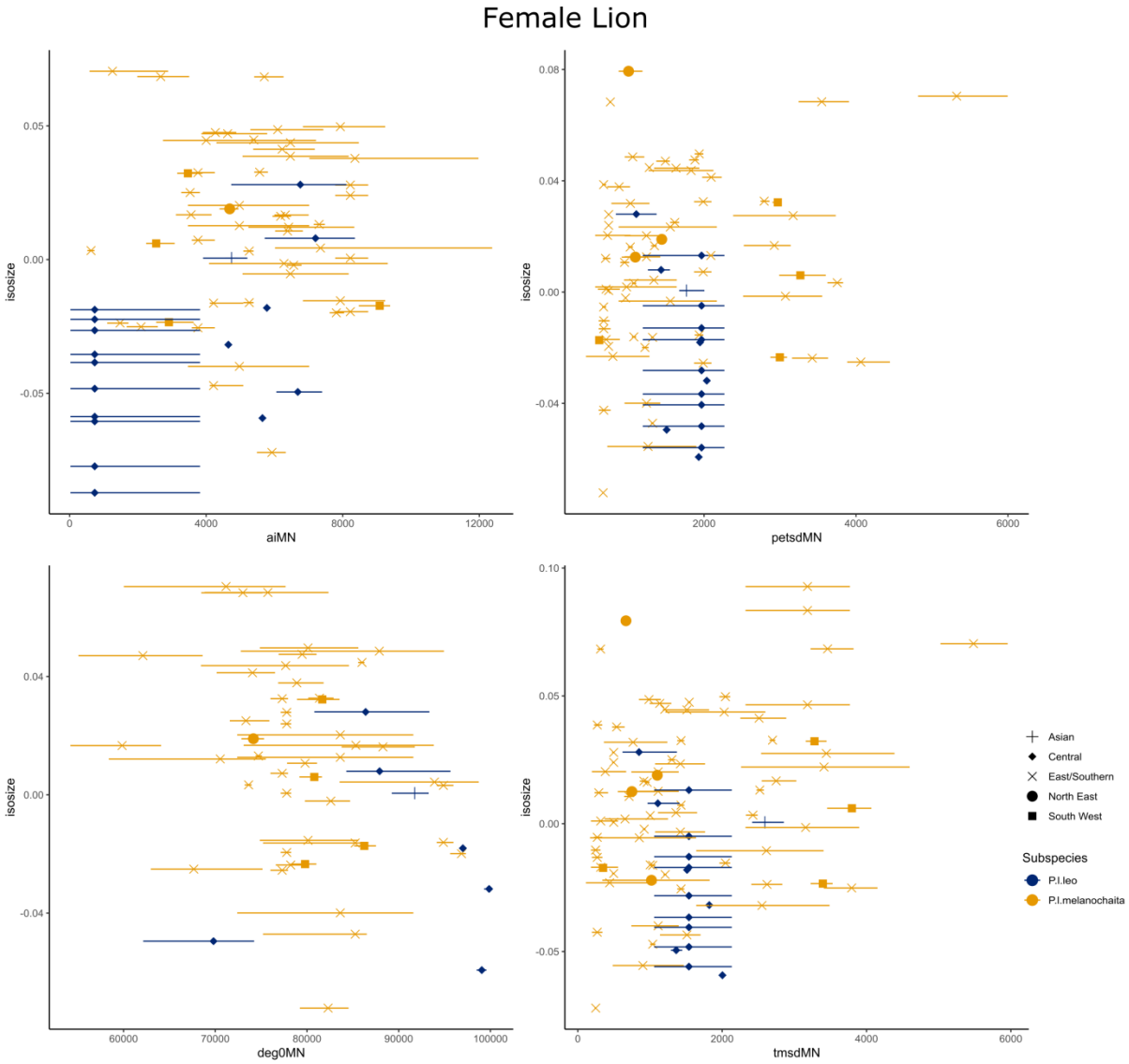


Figure 9: The relationship between bioclimatic variables and isometric size in female lions. Error bars display the climatic uncertainty of each specimen given their textual locality descriptions. Specimens with very high climatic uncertainty have been removed.

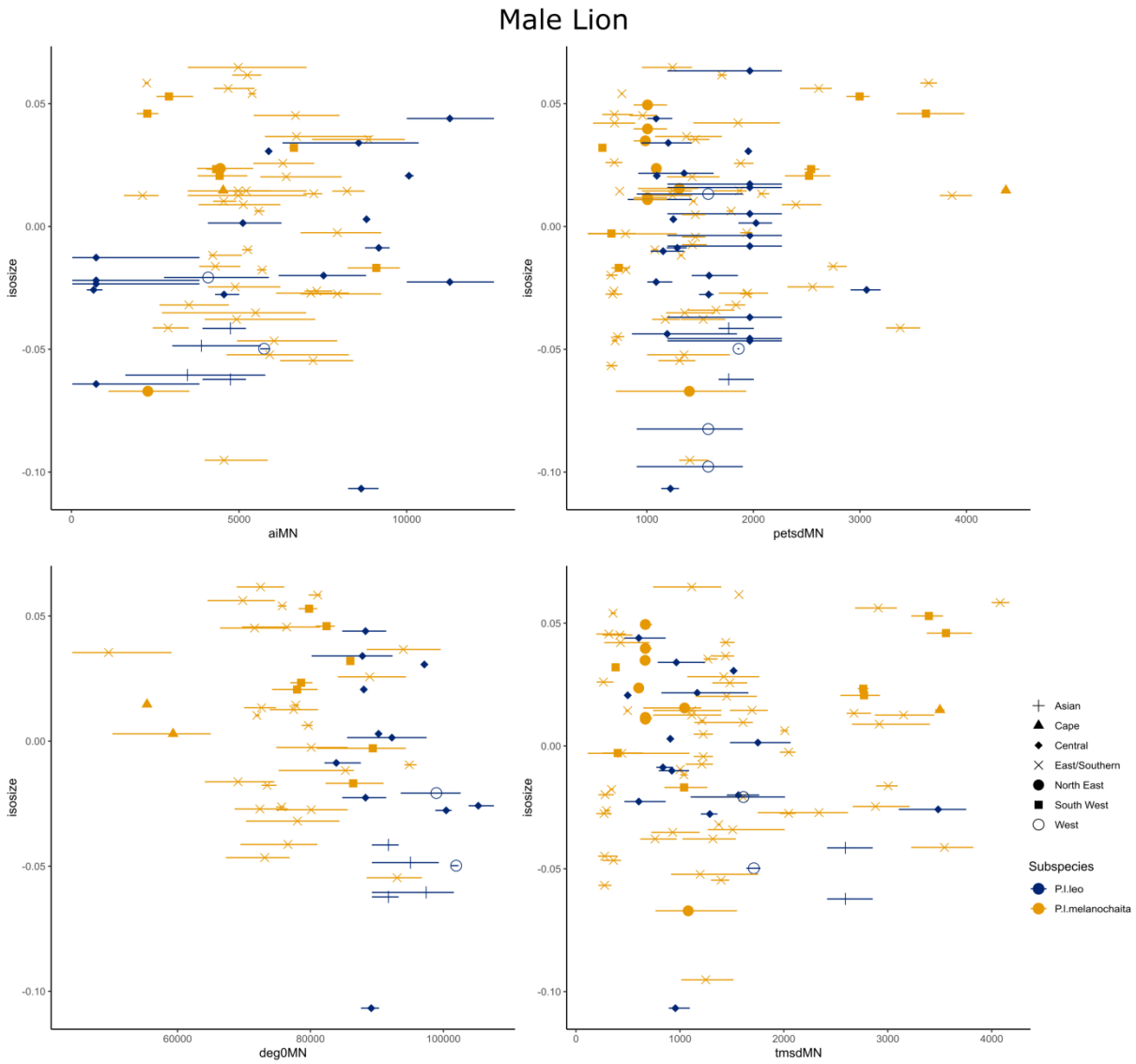


Figure 10: The relationship between bioclimatic variables and isometric size in male lions. Error bars display the climatic uncertainty of each specimen given their textual locality descriptions. Specimens with very high climatic uncertainty have been removed.

Figures 11+12 highlight the vastly different bioclimatic conditions of the Sunda Island tigers from Continental tigers, and from Amur tigers from other Continental tigers. Isosize does not respond to bioclimatic conditions in a linear fashion, highlighted by the very similar environmental conditions found by Sunda Island tigers, despite considerable variation in body size.

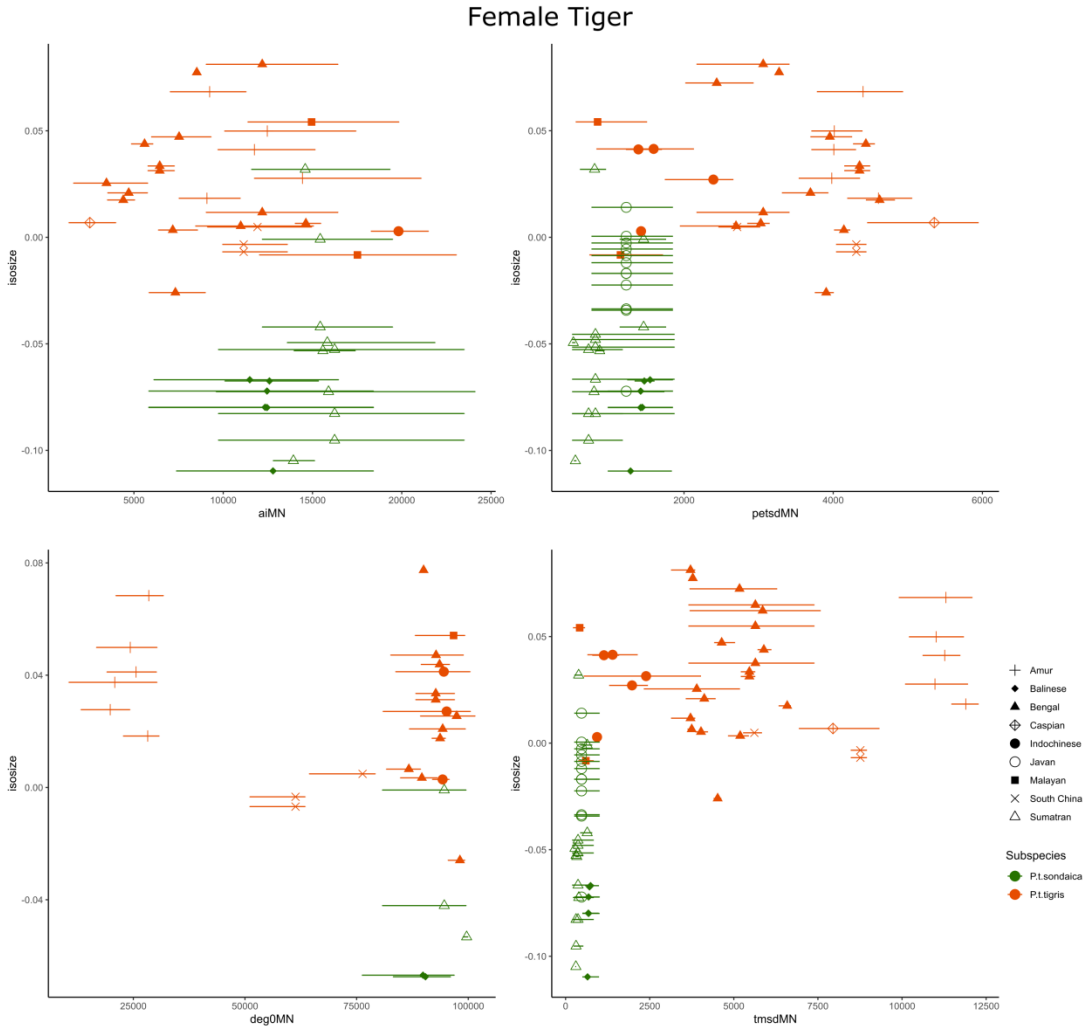


Figure 11: The relationship between bioclimatic variables and isometric size in female tigers. Error bars display the climatic uncertainty of each specimen given their textual locality descriptions. Specimens with very high climatic uncertainty have been removed.

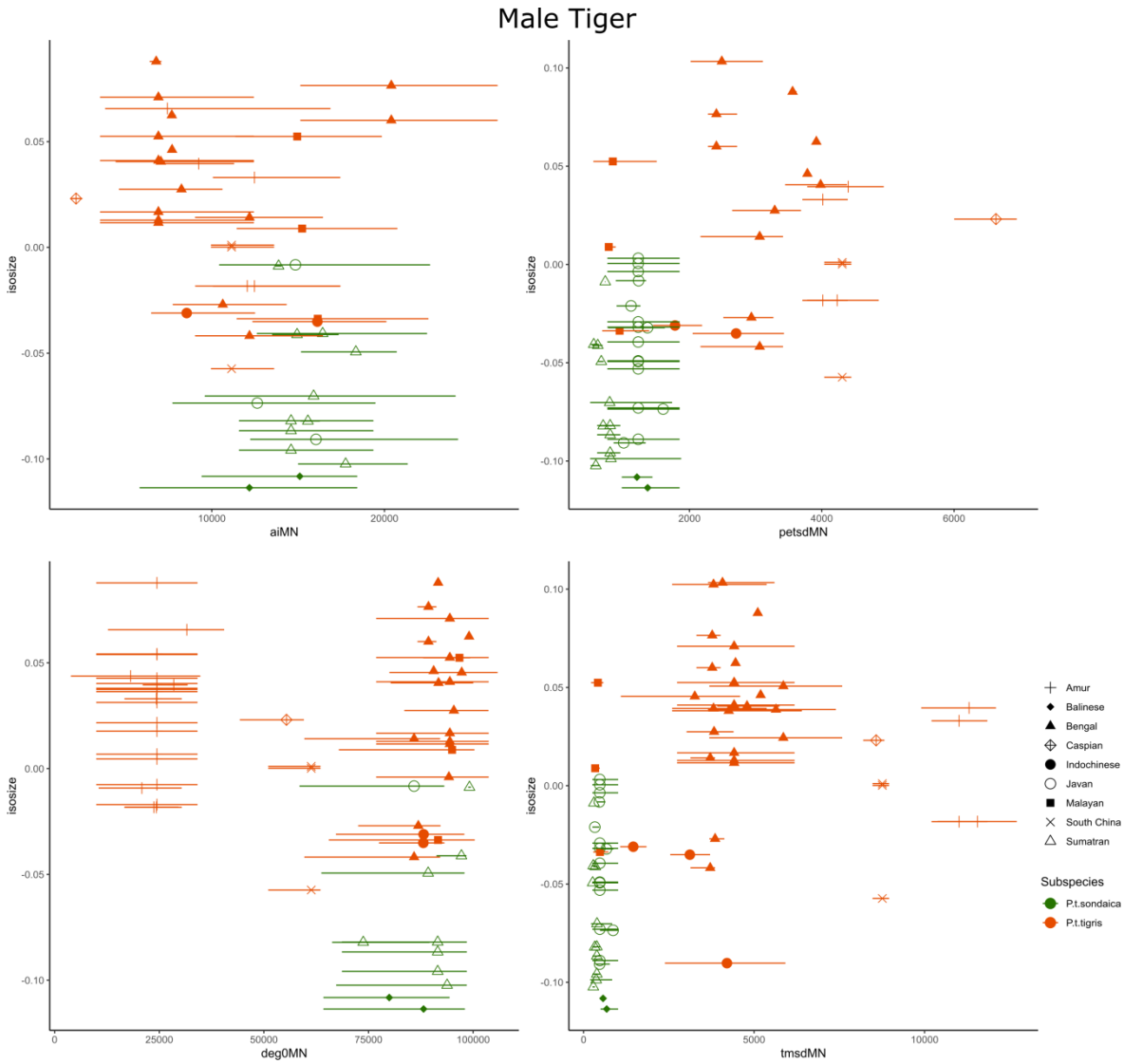


Figure 12: The relationship between bioclimatic variables and isometric size in male tigers. Error bars display the climatic uncertainty of each specimen given their textual locality descriptions. Specimens with very high climatic uncertainty have been removed.

Discussion

Skull size in the lion and tiger

The skull size of the lion and tiger shows variation throughout their respective ranges. However, there is considerable variation between clades and subspecies of each species, and no clear step change in size by any proposed grouping. As such it is unlikely that long-term vicariance of populations has led to variation in body size. Whilst body size can change in response to environmental conditions (Teplitsky & Millien, 2014), there is no discernible relationship between skull size and captivity status (*Chapter 5*). Given that size has been shown to change rapidly within populations (Greenberg et al., 1998; Gardner et al., 2011; Tomassini et al., 2014), it is likely that size in the lion and tiger is a result of short-term selective pressure under prevailing environmental conditions.

The latitudinal scaling of skull size found in both the lion and the tiger does not conform well to a single biogeographical rule dictating size. The results here show that skull size increases from north to south through the range of the lion, which bisects the equator. This pattern does not strictly conform to Bergman's Rule which would dictate that equatorial lions should be smaller than those found at increasing latitude both north and south. Whilst there is a general north-south trend in skull size, there is considerable variation. Lions from equatorial regions, where they live in open, prey-rich habitats (Lorenzen et al., 2012), cover the total size range of all individuals in both males and females (however this is also where number of wild specimens is largest).

The clinal patterns of variation in size and shape between geographical groupings in the tiger is well known (Kitchener, 1999), yet the driving mechanisms are complicated by multiple selective pressures upon body size. In the southern part of the tigers range, the size of the islands they occupy progressively increases, from Bali, Java to Sumatra and the Mainland. However, skull size in the tiger does not progressively increase, as the Javan tiger is larger than those of tigers found on Sumatra in both male and female specimens. It is possible that the more open habitats of Java compared with the dense tropical rainforests of Sumatra (Meijaard, 2004) have favoured larger body size, and cranial size in the tiger. Dense habitats have been shown to correlate with smaller body sizes in bovids due to the Manoeuvrability Hypothesis (Bro-jørgensen, 2018). Tigers may be smaller within denser habitats to be more

manoeuvrable, because prey species are smaller, or both. Habitat density is difficult to test against in this regard, as global datasets of tree density do not account for the physical structure of the understorey which may hinder movement and select for smaller body sizes. The effects of island dwarfing in large carnivores are complicated by multiple influencing factors, and external processes may obscure overall trends (Meiri et al., 2005). The critical size of island to affect body size may be smaller than the size of either Sumatra or Java, and this could explain the much smaller skull size of the Bali tiger compared with the Javan tiger, which both occupy near identical latitudes, geographical and phylogenetic proximity and bioclimatic conditions. The results show that skull size does not greatly increase in the tiger beyond the Malay Peninsula, despite a large disparity in latitude and bioclimatic conditions between the Amur tiger and other Continental tigers. This shows that coarse-scale bioclimatic data do not well explain variation in skull size of the tiger. The results presented here show that bioclimatic conditions follow a stark change between the Sunda Islands/ Peninsula Malaya and the rest of the Continental range of the tiger, yet skull size exhibits a more gradual increase. It is possible that decreasing gene flow through the Malay Peninsula has prevented a step change in body size selected by environmental conditions, because even low rates of gene flow can inhibit local adaption under strong selection (Postma & Noordwijk, 2005). The narrow linear nature of the Malayan peninsular may also effectively act like an island, becoming progressively less constrained as it connects to the rest of the continent in the north.

Skull shape in the lion and tiger

The lack of shape differentiation (beyond that associated with size) between different clades in the lion is consistent with the contiguous potential range of the lion as found in *Chapter 4*, although is discordant with the very long-term genetic splits found by Bertola et al., (2016) and with recognised subspecies of the lion (Kitchener et al., 2017). In wild lions, skull growth is subject to variation between individuals from neighbouring prides or from the same prides as well as between different populations (Smuts et al., 1978), mainly believed to be due to different dietary characteristics. It is therefore possible that the lack of differentiation of size and shape between clades is caused by high variation in the prey preferences and strategies of individual prides. Even within pride variation may occur due to the hierarchy of the individual, time spent hunting, and level of dominance over a carcass in a group feeding

setting. The Cape lion is not found to be morphologically distinct from other East/Southern lions, which supports most existing genetic and morphological studies (Barnett et al., 2006; Mazak, 2010). A previous analysis which proposed the distinctiveness of the Cape lion (Christiansen, 2008) applied linear discriminant analysis to a very small sample size, and thereby inflated variation in individuals which has promoted the apparent distinctiveness of Cape lions. Asian lions differ in shape from other populations of the lion. The Asian lion has been through a severe population bottleneck and is highly inbred (Shankaranarayanan et al., 1997), and so it is possible that some shape differentiation is due to the unique genetic composition of this population. The differentiation between Asian lions and other lions occurs in measurements that are shown to be highly plastic, and in a pattern consistent with differentiation in masticatory action. The results show that the bioclimatic conditions found within the Asian range of the lion are considerably different from those of African lions and so the clear discordance in shape between Asian and African lions is likely due to their respective environments and prey composition. This is further supported by the similarity in skull shape of West African lions, which are genetically similar to Asiatic lions, compared to Eastern and Southern lion clades. This study would benefit from a greater number of lion specimens from North Africa and the Near East, as no clinal relationships from Africa to Asia are testable with the available data. As these populations have been extirpated from the wild and are scarce in museum collections, it is unlikely to be possible to test this trend.

Clinal variation in shape in the tiger is correlated with skull size, and therefore likely represents allometric scaling. Whilst sPC1 is strongly associated with skull size, and increases through the range of the tiger, there is also clear separation of Amur tigers from other similarly sized Continental tigers on the same axis. Previous craniometric analysis has also shown that the Amur tiger as the most distinct of the mainland Asia tigers, whilst Indian, Indochinese and South China tigers show clear morphological overlaps (Mazák, 2010). The difference in skull parameters between Amur tigers and other Continental tigers mirrors the differences between wild Amur tigers and captive Amur tigers. Wild Amur tigers have generally narrower skull proportions than both Amur tigers in captivity and other wild Continental tigers. The sagittal crest has been found to be more developed in the Amur and Caspian tiger than in other Continental tigers (Kitchener & Yamaguchi, 2010). Wild Amur tigers have greater cranial volumes than other Continental tigers, which again follows the pattern of greater ‘wildness’ in Amur tigers than other Continental tigers. The difference in cranial volume between wild tiger and lion populations, which follows the same patterns as

other captive/wild disparities in part dispels the notion that differences in cranial volume in captivity are pathological (Howell, 1917). The increased cranial height and sagittal crest length of Amur tigers, and decreased skull widths compared with other Continental tigers is likely a plastic response to the vastly different environmental conditions of the Russian Far East, compared to the rest of the Continental tigers range. It is likely that the prey composition and preferences of the Amur population has promoted increased masticatory action when compared with other Continental tigers. This conclusion is further supported by the available Caspian specimens, which are genetically most similar to Amur tigers (Driscoll et al., 2009; Liu et al., 2018) yet are morphologically more similar to other Continental tigers (although the sample of Caspian tigers is very small). Whilst patterns between Amur and other Continental tigers generally follow patterns between captive and wild specimens, it should be noted that mandible heights in Amur tigers is lower other Continental tigers, which goes against this trend and there is significant differentiation in the postorbital bar which is not discriminatory between wild and captive tigers. It is possible that the geographical dissociation of the Amur populations from southern populations since ~8ka (*Chapter 3*) has led to heritable characteristics of skull shape of this population. Similarly to shape differentiation between Asian lions and African populations, it would be expected that a more clinal relationship between the morphology of Amur tigers and other Continental tigers would be found with a greater number of specimens from mainland China. However, these specimens are not available.

Shape differentiation between the Javan and Sumatran populations is pronounced in sPC3, especially between females. There are apparent step changes in variation in shape between the Sunda Island tiger populations, especially in females, yet Continental tigers broadly cover the range of shape principal component values of Sunda Island tigers. Assessing variation for each measurement is difficult because the sizes of specimens between the Sunda Islands are not comparable, and therefore variation in measurements may be affected by skull size despite using scaled variables. By splitting the data across each Sunda Island population, the sample sizes are also limited. Regardless of these shortcomings, shape differences between the Sunda Island populations do not follow patterns of variation found between captive and wild specimens. It is likely that shape differences between Sunda Island populations represent a combination of both allometric scaling due to size disparities, and the stochastic fixation of genes (Sicuro & Oliveira, 2011) following their isolation from the mainland and from each other (*see Chapter 3*).

Populations which exhibit clinal variation should only legitimately be subdivided into subspecies if discontinuities or step clines occur (Winker, 2010). Traditionally, populations have been considered as separate subspecies if 75% of the population's morphological characteristics lie outside of 99% of the range of other populations (Amadon, 1949), however it is argued a subspecies should be diagnostically distinct (Torstrom et al., 2014). It should be noted that skull measurements are only one aspect of felid morphology, and that important variation may exist within the postcranial skeleton. The results reported here show that step changes in morphology can occur between isolated populations due to phenotypic plasticity, and that known long-term evolutionary splits can occur without clear morphological differentiation. Anatomical variation within the family Felidae is small, with the exception of body size (Randau *et al*., 2016). Strong relationships between geographical regions and morphology are shown to be stronger in species with high habitat specialisation (Jonsson & Jonsson, 2001; Langerhans et al., 2003). Considering that both lions and tigers have wide environmental tolerances, this may have limited the ecogeographical signal found. It is likely that even low rates of gene flow between populations have prevented step changes in morphological variation. This is supported by the maintained differentiation of Sunda Island populations where gene flow has been mostly prevented by island isolation.

Conclusions

This chapter investigates wild skull morphology for the lion and tiger in relation to potential evolutionary, and life-history drivers. Whilst clinal size variation is found in both species, there is no step change in size by any proposed taxonomic grouping, and it is likely that size variation occurs due to a combination of environmental factors operating within different geographic regions and at different spatial scales. The results presented here do not find any single bioclimatic factor explaining the variation in skulls of the lion or the tiger, however a comparison of skull size with bioclimatic data highlights the disparity of environmental conditions between certain populations, such as between the Amur tiger and other Continental tigers.

Differentiation of the Amur tiger from other Continental tigers, and the Asian lion from other lions is broadly consistent with measurements which differ between captive and wild specimens. It is therefore likely that the vastly different environmental conditions of their now isolated ranges reflects most of their morphological distinctiveness rather than differentiation through evolutionary divergence. There are step changes in the skull morphology between the Sunda Island tigers which are likely due to a combination of allometric scaling, and geographical isolation and the random fixation of genes through drift. Conversely, a lack of step change between established taxonomic groupings is likely caused by a combination of comparable environmental conditions and gene flow between neighbouring populations. The Cape lion is not found to be morphologically distinct from other East/Southern lions.

The results here point to the value of using captive-bred animals for comparison with wild animals in order to determine which skull characters vary due to phenotypic plasticity or genetic determination. Thus, apparent taxonomic differences due to phenotypic plasticity can be negated by such analyses and instead focussed on characters that are likely due to genetic differences between populations.

Supplementary Information

Table S1: t-test p-values for scaled measurements between female (FL) and male (ML) Asian and other Northern lions, and between female (FT) and male (MT) Amur and other Continental tigers. p-values below 0.0009 Bonferroni correction are highlighted in red.

	FL Asian:North.	ML Asian:North.	FT Amur:Cont.	MT Amur:Cont.
Cranial volume (ml)	0.0117	0.0116	0.0000	0.0001
Frontal breadth	0.2575	0.2987	0.0085	0.0000
Greatest length	0.1731	0.9809	0.0050	0.9349
Condylobasal length	0.0062	0.0283	0.0000	0.0018
Palate-inion	0.0174	0.0181	0.1159	0.0672
Nasal-inion	0.0274	0.6031	0.2015	0.0386
Facial length	0.8912	0.6922	0.0152	0.0009
Head length	0.1198	0.2678	0.6321	0.7609
Bizygomatic breadth	0.0739	0.7627	0.8881	0.3099
Zygomatic length	0.2935	0.6935	0.2578	0.3264
Zygomatic length anterior	0.1463	0.1249	0.0007	0.2163
Zygomatic length posterior	0.0335	0.2235	0.5093	0.8713
Orbit vertical	0.0853	0.4775	0.0026	0.0002
Orbit horizontal	0.4543	0.0171	0.5598	0.8815
Postorbital bar	0.0249	0.0127	0.0000	0.0037
Facial length anterior	0.8323	0.5751	0.8847	0.0811
Facial length posterior	0.7535	0.2600	0.0008	0.0000
Sagittal crest	0.1380	0.8094	0.0251	0.0000
Cranial height-I	0.8118	0.0367	0.0004	0.0000
Cranial height-II	0.9114	0.0290	0.0000	0.0000
Cranial height-III	0.1366	0.0032	0.0000	0.0000
Cranial height -IV	0.6151	0.9438	0.0000	0.0053
Interorbital breadth	0.4480	0.0006	0.0000	0.0000
Postorbital breadth	0.0006	0.0000	0.0000	0.0094
Nasal length-I	0.1127	0.0000	0.0000	0.0000
Nasal breadth	0.7784	0.0014	0.6689	0.9437
Breadth between infra orbital foramina	0.2232	0.8399	0.0001	0.0175
Rostral depth-I	0.1060	0.0000	0.3337	0.3872
Rostral depth-II	0.0608	0.0000	0.1599	0.9632
Rostral breadth	0.0263	0.0056	0.0093	0.0296
Nasal aperture	0.0158	0.0023	0.0000	0.0000
Upper jaw	0.9538	0.0294	0.0000	0.0050
Palate length	0.0175	0.0947	0.0052	0.0056
Palate breadth-I	0.0020	0.1802	0.6202	0.0256
Palate breadth-II	0.6376	0.3247	0.0000	0.0000
Canine - Pm^4 (alveolus - alveolus)	0.9678	0.0490	0.0489	0.0607
Pm^2 - Pm^4 (alveolus - alveolus)	0.2235	0.1584	0.4326	0.0317
Pm^4 length	0.7593	0.0001	0.5255	0.0056
Mastoid breadth	0.0749	0.0005	0.0000	0.0000
Skull height-I	0.9233	0.5170	0.0010	0.5971
Skull height-II	0.9520	0.1269	0.0007	0.0633
Foramen magnum height	0.0202	0.0000	0.3876	0.7046
Occipital condyles breadth	0.3832	0.0559	0.0000	0.0000
Mandible length	0.0553	0.1508	0.3435	0.3639
Mandible length coronoid process	0.0491	0.0682	0.1691	0.0045
Mandible length angular process	0.2153	0.0952	0.4765	0.8081
Mandible height	0.4349	0.0487	0.0016	0.0849
Mandible height angular process	0.3326	0.0760	0.0069	0.0353
Mandible height coronoid process	0.6454	0.2710	0.0002	0.8455
Width of the mandibular condyle	0.0149	0.0013	0.3140	0.9957
Mandible depth-I	0.8484	0.0800	0.0143	0.0009
Mandible depth-II	0.8064	0.0718	0.0009	0.0000
Canine - M^1 (alveolus - alveolus)	0.6128	0.5723	0.3864	0.0071
Pm^3 - M^1 (alveolus - alveolus)	0.0233	0.0051	0.0006	0.0044
Pm^4 length	0.3277	0.3417	0.2093	0.6175
M^1 length	0.5463	0.0055	0.0000	0.0000

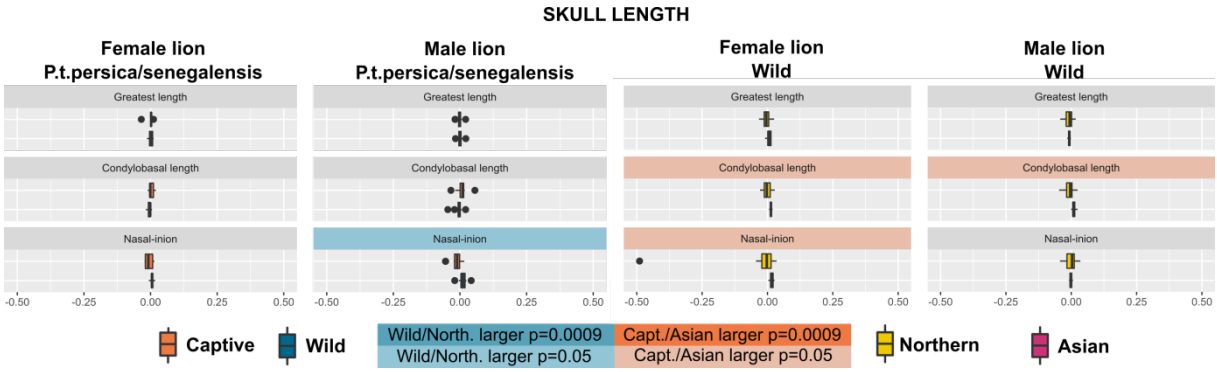


Figure S1: (LEFT): Variation of size independent (scaled) variables by captivity status for measurements representing skull length for northern lions. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. (**RIGHT**): Variation in variables between Asian, and other northern lions. Measurements which differ significantly are highlighted, using the same colour scheme as between captive and wild specimens to aid interpretation. Significance is determined using t-tests (*Table S1 + Chapter 5 Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.**

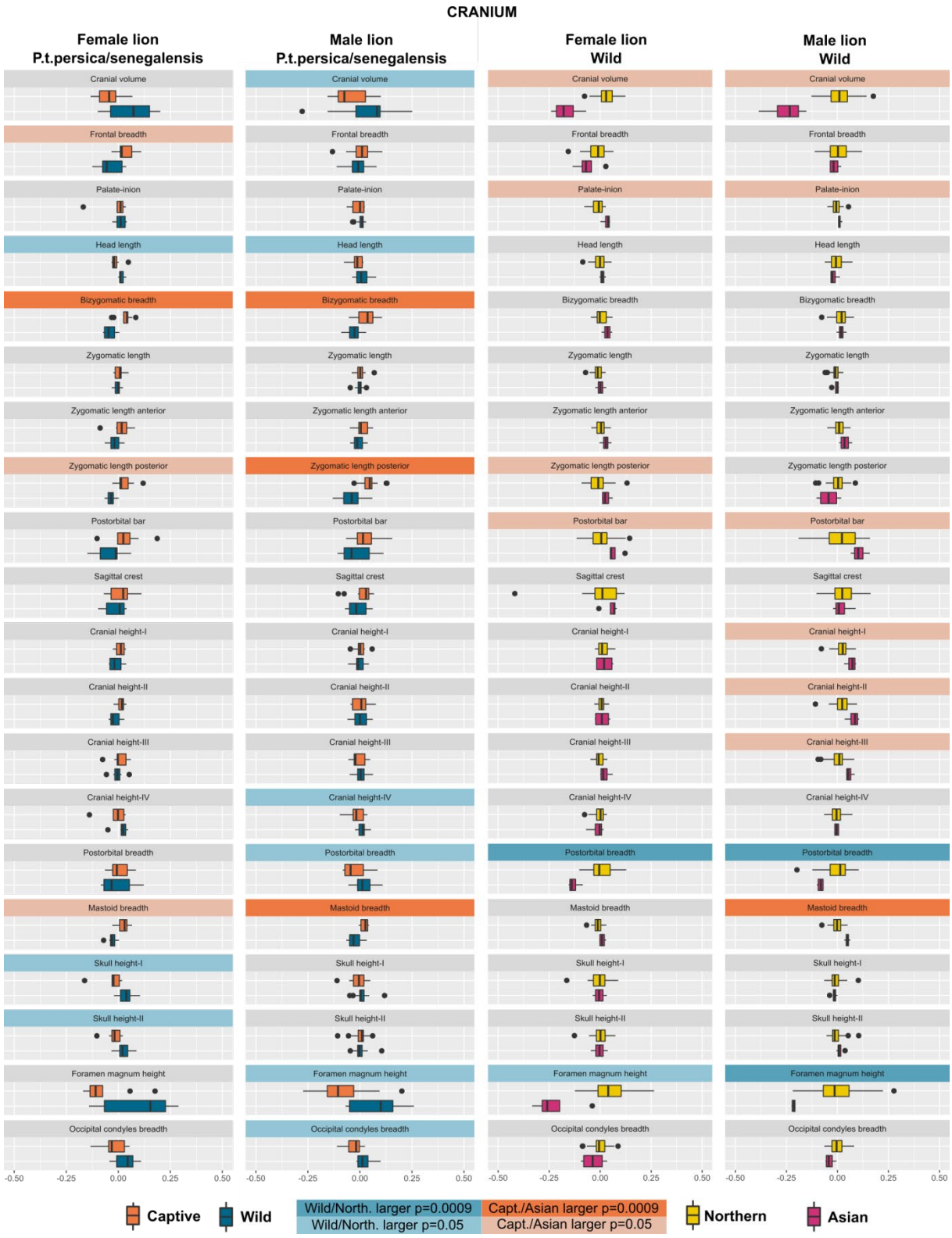


Figure S2: (LEFT): Variation of size independent (scaled) variables by captivity status for measurements representing the neurocranium for northern lions. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. (**RIGHT**): Variation in variables between Asian, and other northern lions. Measurements which differ significantly are highlighted, using the same colour scheme as between captive and wild specimens to aid interpretation. Significance is determined using t-tests (*Table S1 + Chapter 5 Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.**

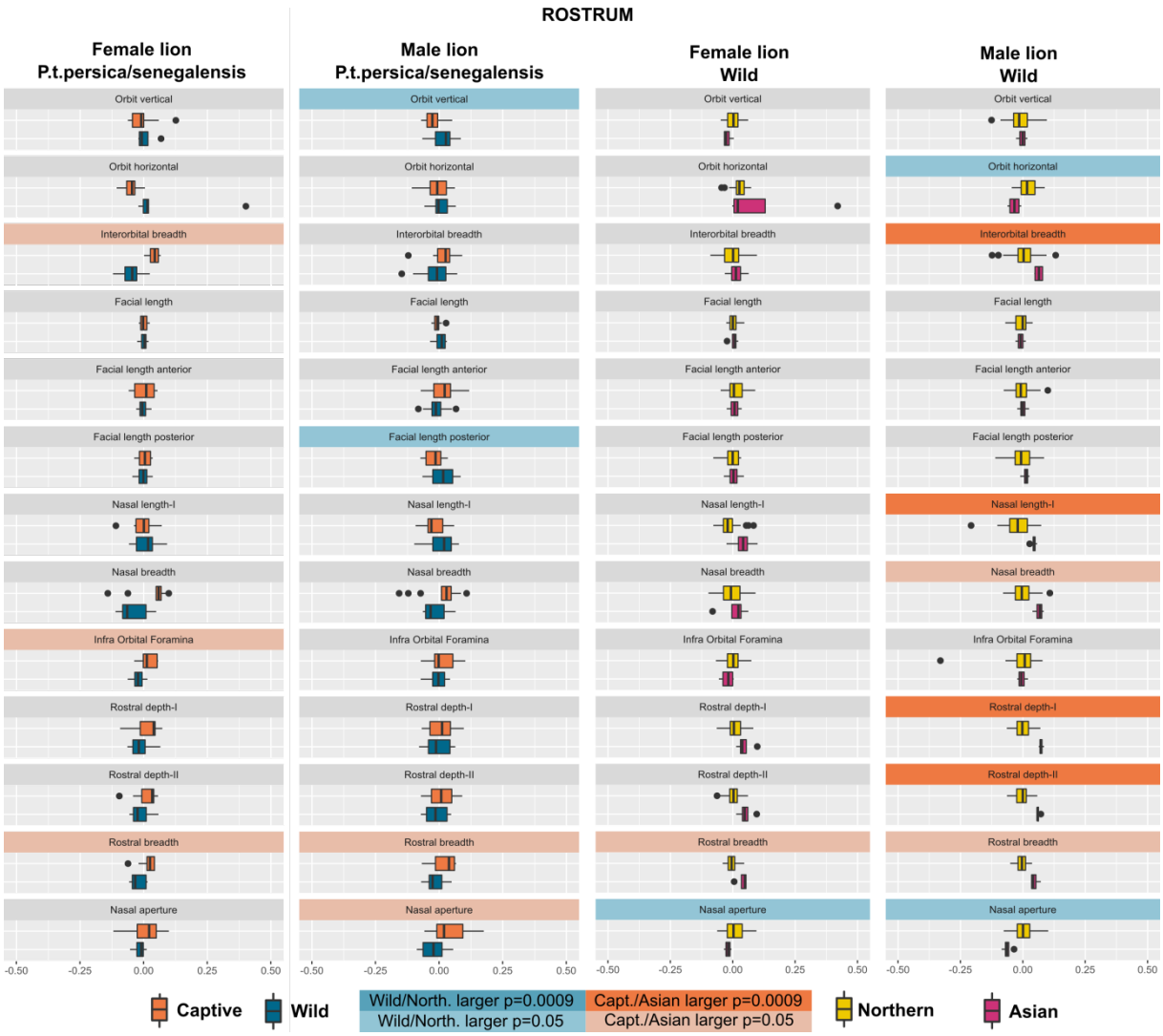


Figure S3: (LEFT): Variation of size independent (scaled) variables by captivity status for measurements representing the rostrum for northern lions. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. (**RIGHT**): Variation in variables between Asian, and other northern lions. Measurements which differ significantly are highlighted, using the same colour scheme as between captive and wild specimens to aid interpretation. Significance is determined using t-tests (*Table S1 + Chapter 5 Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.**

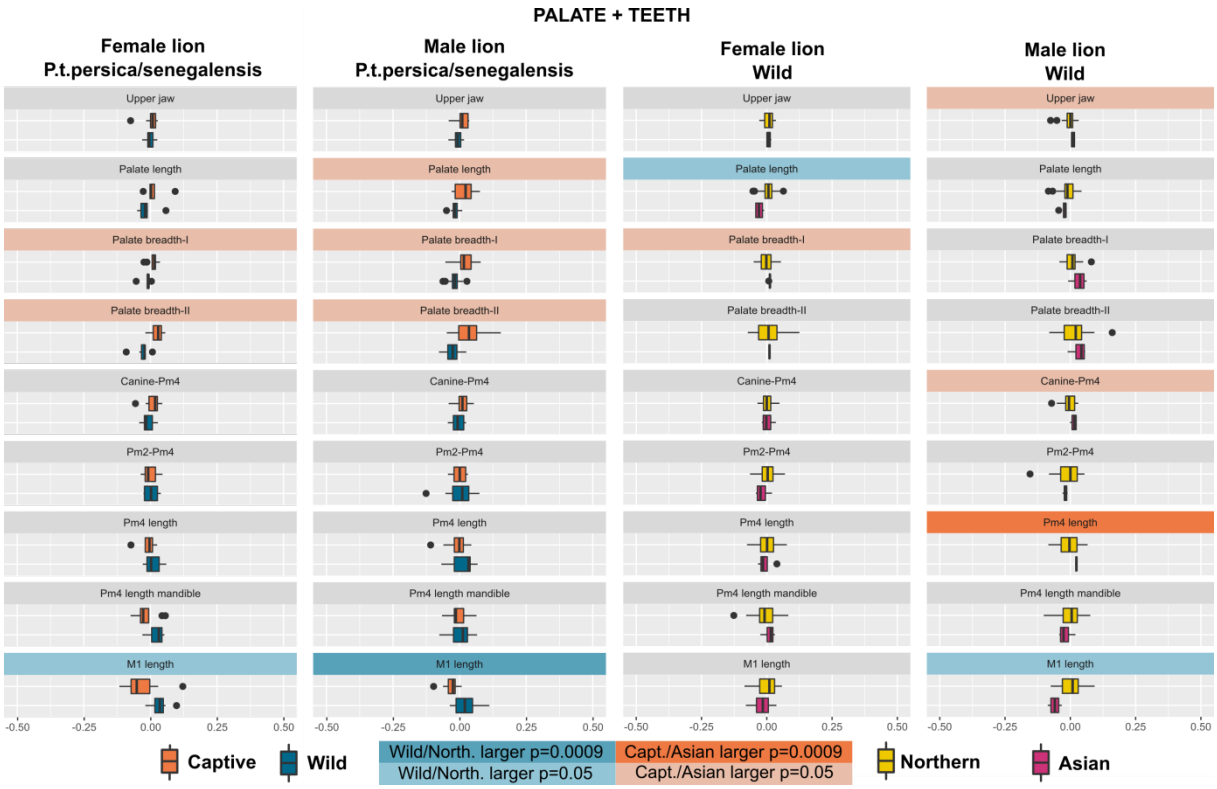


Figure S4: (LEFT): Variation of size independent (scaled) variables by captivity status for measurements representing the palate and teeth for northern lions. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. (**RIGHT**): Variation in variables between Asian, and other northern lions. Measurements which differ significantly are highlighted, using the same colour scheme as between captive and wild specimens to aid interpretation. Significance is determined using t-tests (*Table S1 + Chapter 5 Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.**

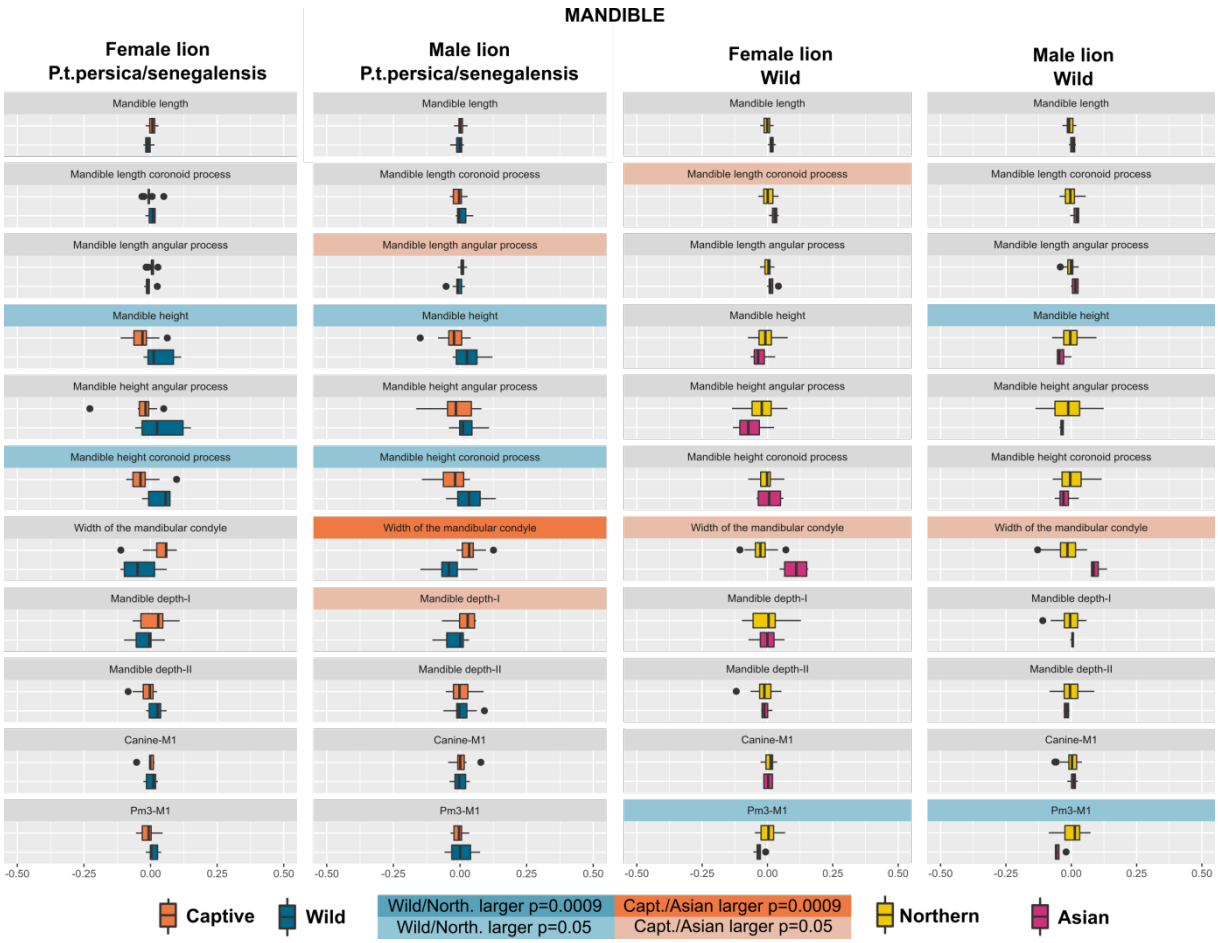


Figure S5: (LEFT): Variation of size independent (scaled) variables by captivity status for measurements representing the mandible for northern lions. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. (**RIGHT**): Variation in variables between Asian, and other northern lions. Measurements which differ significantly are highlighted, using the same colour scheme as between captive and wild specimens to aid interpretation. Significance is determined using t-tests (*Table S1 + Chapter 5 Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.**

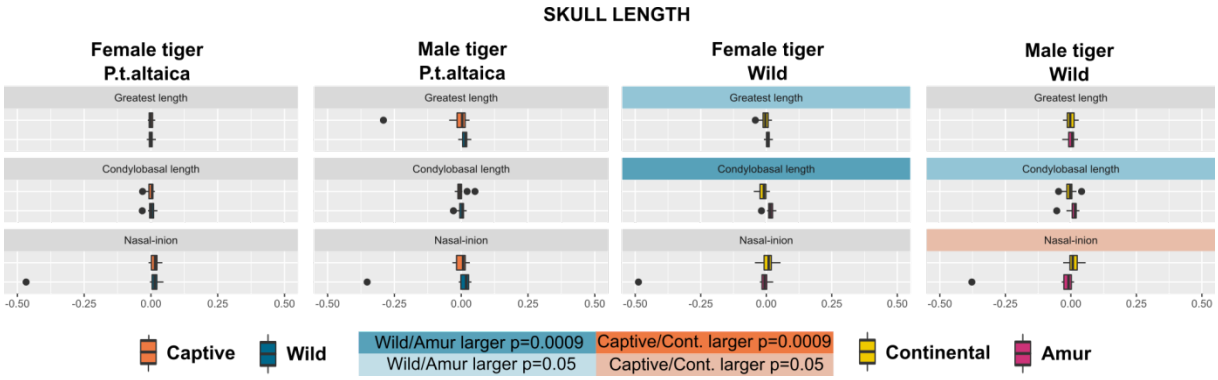


Figure S6: (LEFT): Variation of size independent (scaled) variables by captivity status for measurements representing skull length for Amur tigers. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. (**RIGHT**): Variation in variables between Amur, and other Continental tigers. Measurements which differ significantly are highlighted, using the same colour scheme as between captive and wild specimens, to aid interpretation. Significance is determined using t-tests (*Table S1 + Chapter 5 Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.**

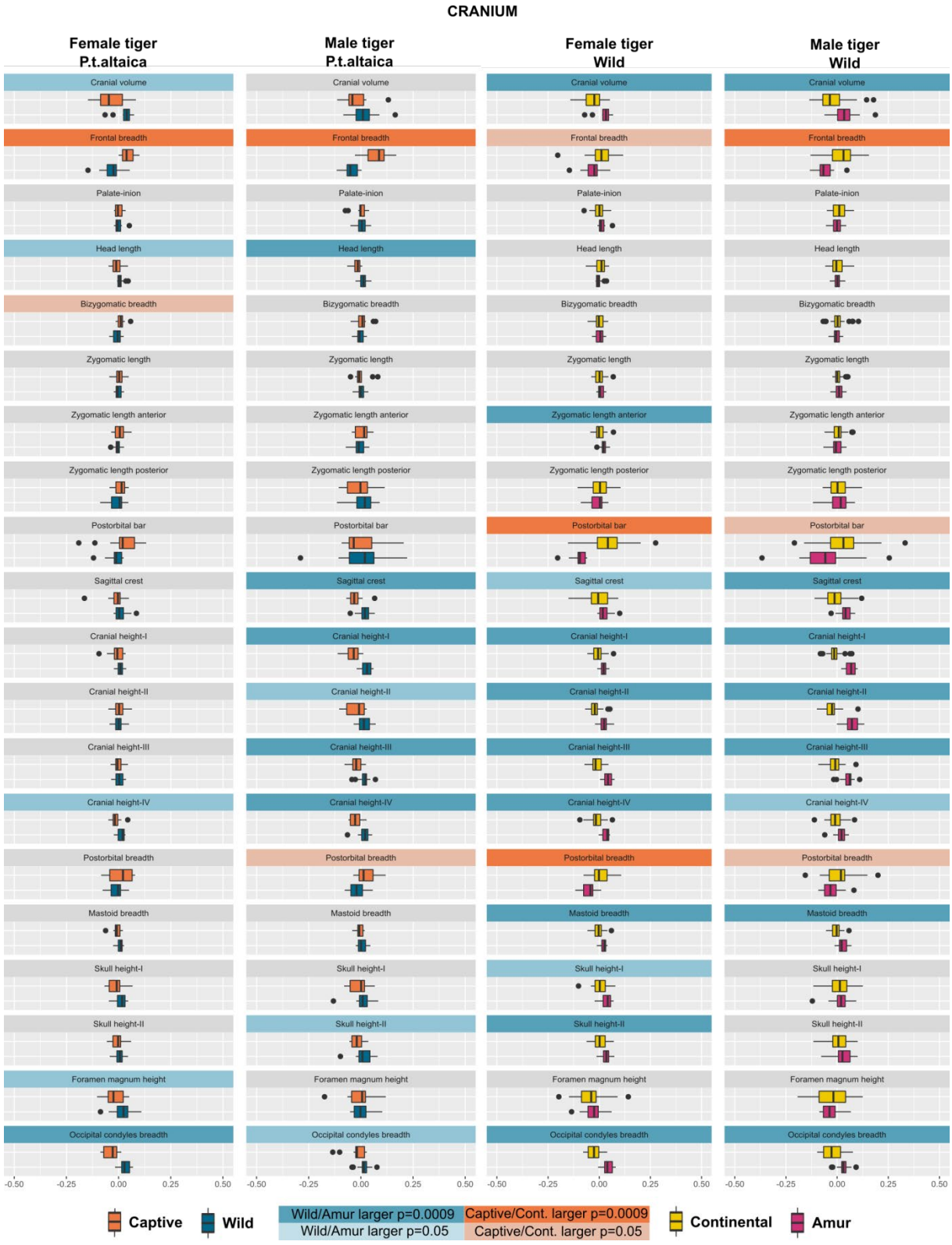


Figure S7: (LEFT): Variation of size independent (scaled) variables by captivity status for measurements representing the neurocranium for Amur tigers. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. (**RIGHT**): Variation in variables between Amur, and other Continental tigers. Measurements which differ significantly are highlighted, using the same colour scheme as between captive and wild specimens, to aid interpretation. Significance is determined using t-tests (*Table S1 + Chapter 5 Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.**

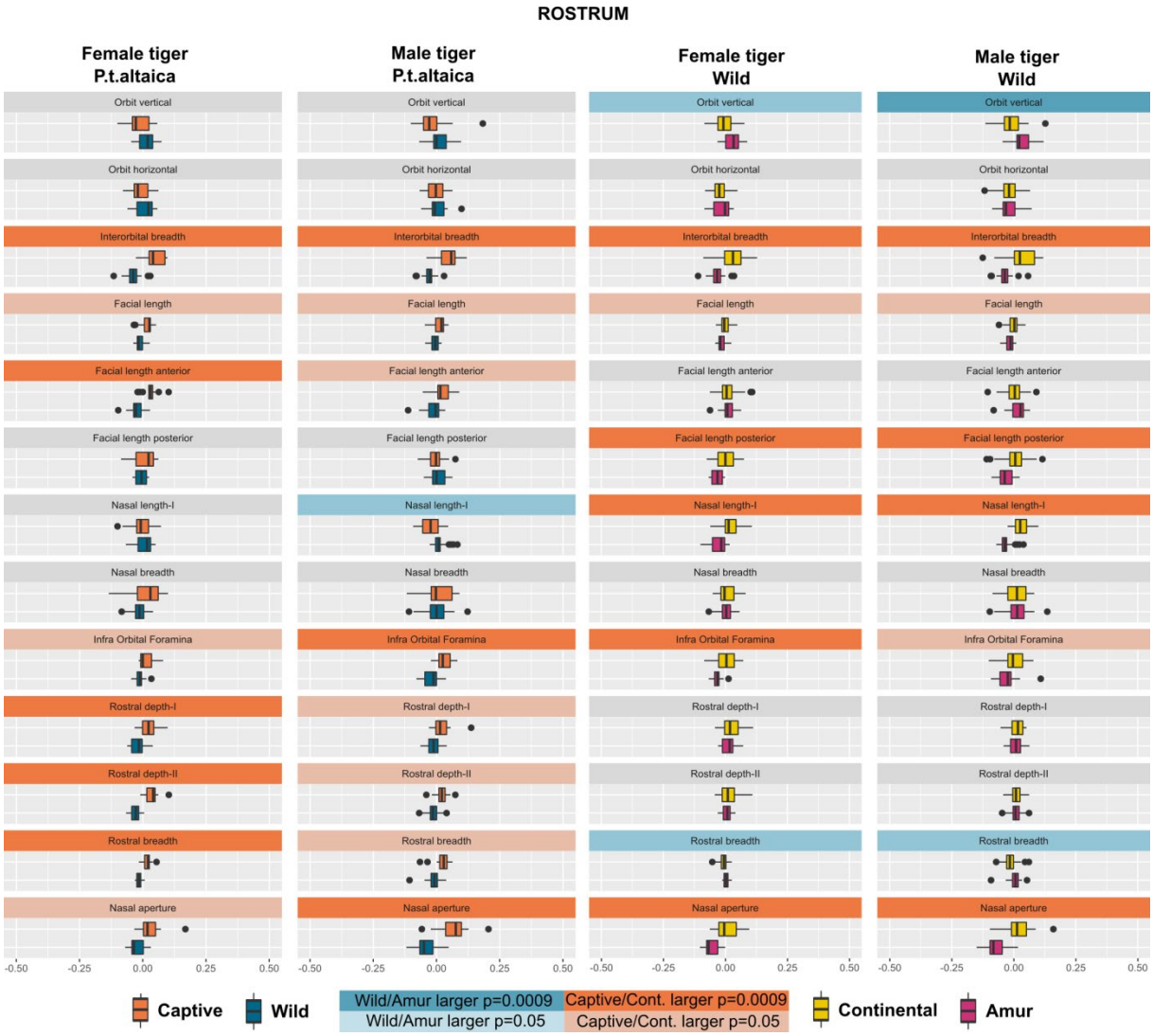


Figure S8: (LEFT): Variation of size independent (scaled) variables by captivity status for measurements representing the rostrum for Amur tigers. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. (**RIGHT**): Variation in variables between Amur, and other Continental tigers. Measurements which differ significantly are highlighted, using the same colour scheme as between captive and wild specimens, to aid interpretation. Significance is determined using t-tests (*Table S1 + Chapter 5 Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.**

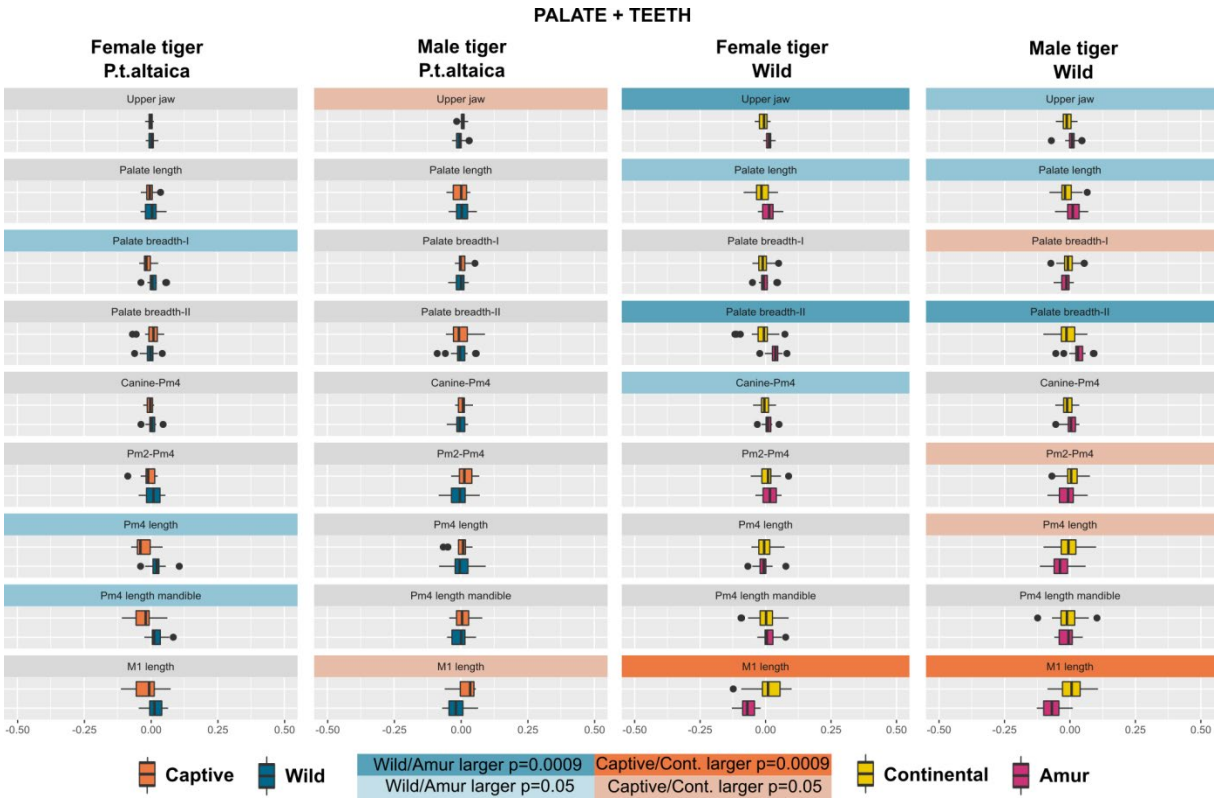


Figure S9: (LEFT): Variation of size independent (scaled) variables by captivity status for measurements representing the palate and teeth for Amur tigers. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. (**RIGHT**): Variation in variables between Amur, and other Continental tigers. Measurements which differ significantly are highlighted, using the same colour scheme as between captive and wild specimens, to aid interpretation. Significance is determined using t-tests (*Table S1 + Chapter 5 Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.**

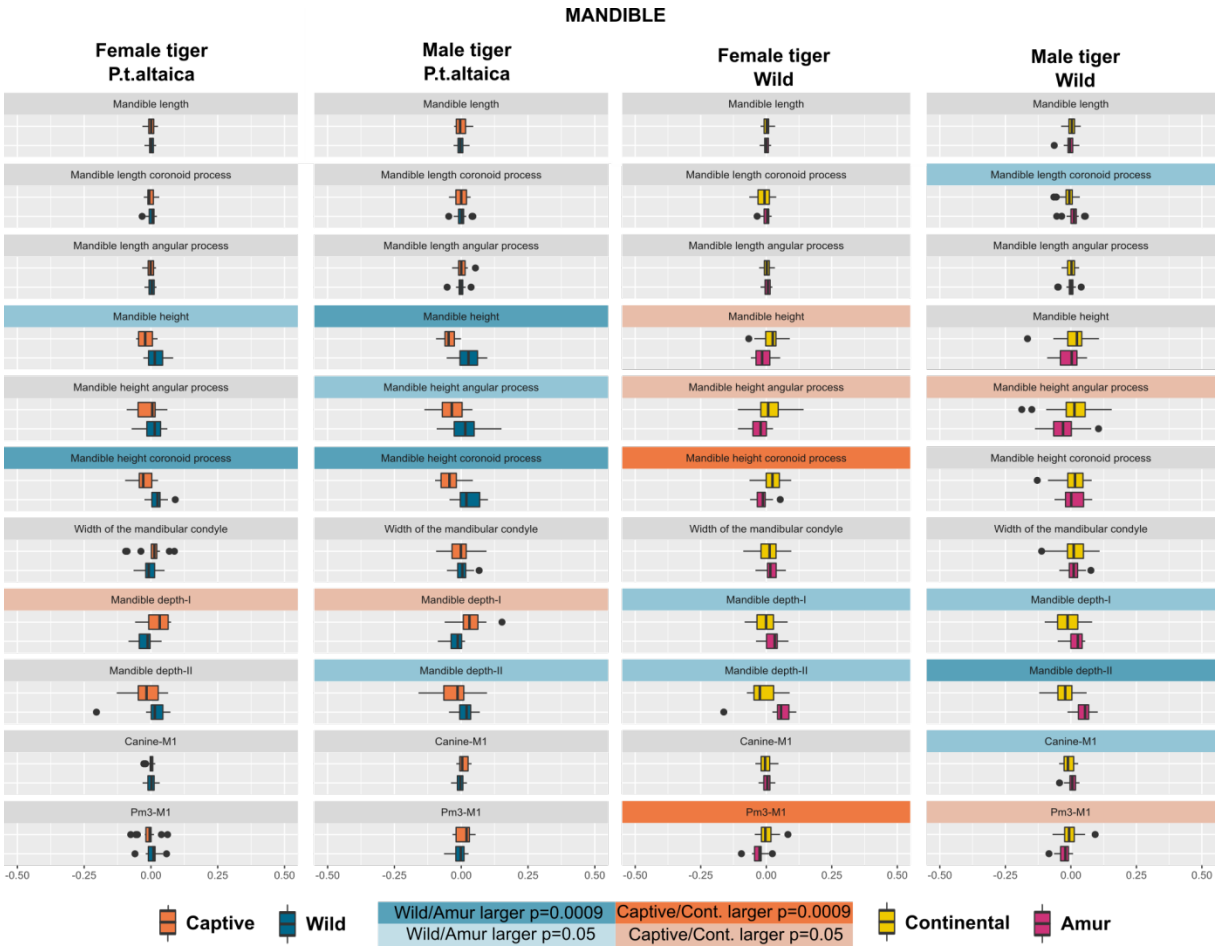


Figure S10: (LEFT): Variation of size independent (scaled) variables by captivity status for measurements representing the mandible for Amur tigers. Measurements which differ significantly by captivity status are highlighted by which population mean is larger. (**RIGHT**): Variation in variables between Amur, and other Continental tigers. Measurements which differ significantly are highlighted, using the same colour scheme as between captive and wild specimens, to aid interpretation. Significance is determined using t-tests (*Table S1 + Chapter 5 Table S2*) based on values of 0.05 and after a Bonferroni Correction, 0.0009. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.**

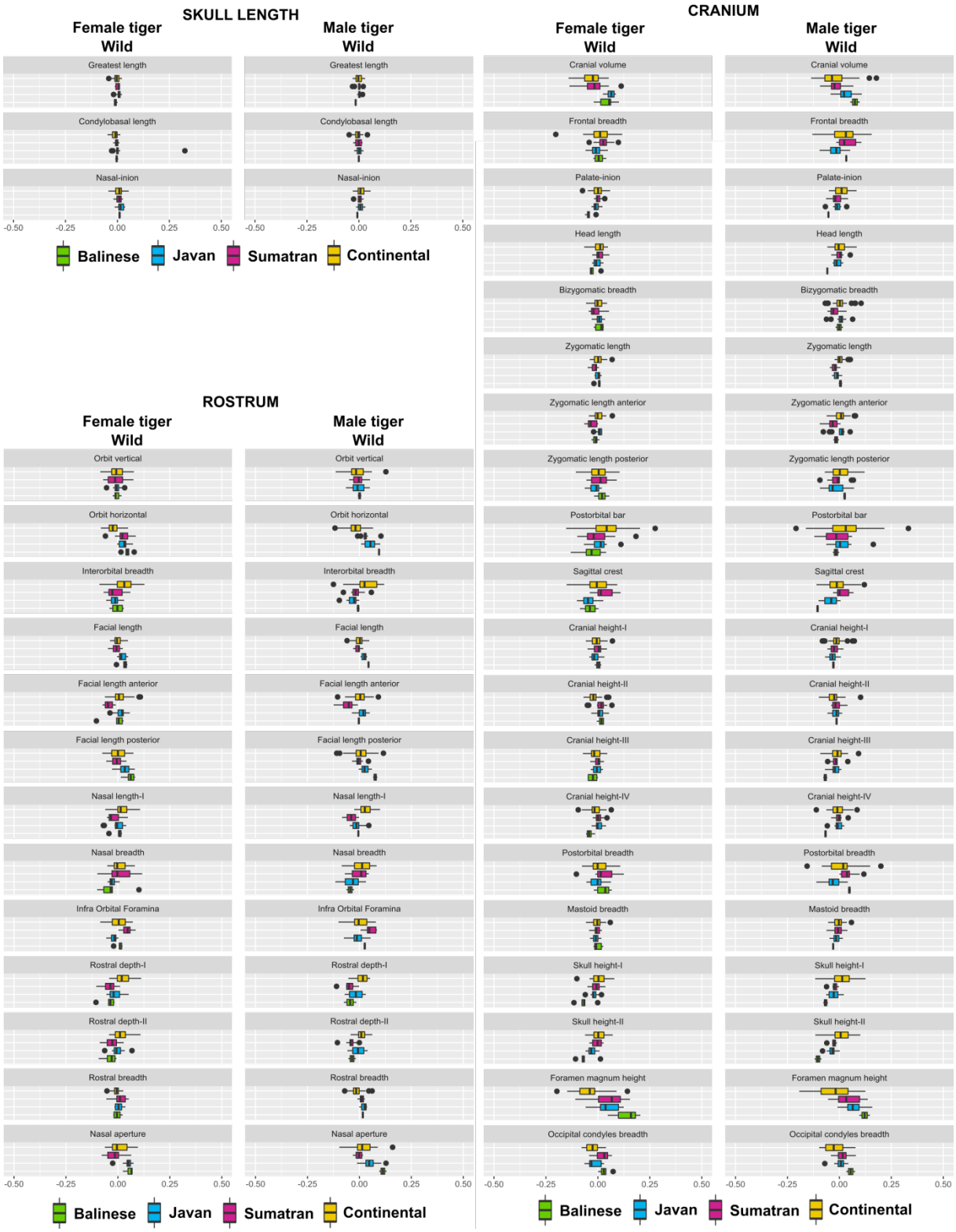


Figure S11: Variation in size independent (scaled) variables between wild Sunda Island tigers, and Continental tigers (excluding the Amur population) for overall skull length, the rostrum and neurocranium. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.**

Figure S12: Variation in size independent (scaled) variables between wild Sunda Island tigers, and Continental tigers (excluding the Amur population) for the palate and teeth and the mandible. Measurements larger than zero are larger than average, and measurements smaller than zero are below average for a given sized skull**.**

References

Amadon D. (1949) The seventy-five per cent. *The Condor*, **51**, 250–258.

Ashton K.G., Tracy M.C., & de Queiroz A. (2000) Is Bergmann's Rule Valid for Mammals? *The American Naturalist*, **156**, 390–414.

Barnett R., Yamaguchi N., Barnes I., & Cooper A. (2006) Lost populations and preserving genetic diversity in the lion Panthera leo: Implications for its ex situ conservation. *Conservation Genetics*, **7**, 507–514.

Barnett R., Yamaguchi N., Shapiro B., & Sabin R. (2008) Ancient DNA analysis indicates the first English lions originated from North Africa. *Contributions to Zoology*, **77**, 7–16.

Bernhard R. (1936) Some problems of geographical variation and species-formation. *The Linnean Society of London*, **150**, 275–285.

Bertola L.D., Jongbloed H., van der Gaag K.J., de Knijff P., Yamaguchi N., Hooghiemstra H., Bauer H., Henschel P., White P.A., Driscoll C.A., Tende T., Ottosson U., Saidu Y., Vrieling K., & de Iongh H.H. (2016) Phylogeographic Patterns in Africa and High Resolution Delineation of Genetic Clades in the Lion (Panthera leo). *Scientific Reports*, **6**, 1–11.

Beyer H.L. (2014) Geospatial Modelling Environment. .

Bro-jørgensen J. (2018) Dense habitats selecting for small body size: a comparative study on bovids. *Oikos*, **117**, 729–737.

Carbone C., Mace G.M., Roberts S.C., & Macdonald D.W. (1999) Energetic constraints on the diet of terrestrial carnivores. *Nature*, **402**, 286–288.

Cardini A. (2003) The geometry of the marmot (Rodentia: Sciuridae) mandible: Phylogeny and patterns of morphological evolution. *Systematic Biology*, **52**, 186–205.

Cardini A., Jansson A.-U., & Elton S. (2007) A geometric morphometric approach to the study of ecogeographical and clinal variation in vervet monkeys. *Journal of Biogeography*, **34**, 1663–1678.

Christiansen P. (2008) On the distinctiveness of the Cape lion (Panthera leo melanochaita Smith, 1842), and a possible new specimen from the Zoological Museum, Copenhagen. *Mammalian Biology*, **73**, 58–65.

Clavel J., Merceron G., & Escarguel G. (2014) Missing data estimation in morphometrics: How much is too much? *Systematic Biology*, **63**, 203–218.

Cooper D.M., Dugmore A.J., Gittings B.M., Scharf A.K., Wilting A., & Kitchener A.C.

(2016) Predicted Pleistocene-Holocene range shifts of the tiger (Panthera tigris). *Diversity and Distributions*, **22**, 1199–1211.

Dayan T. & Simberloff D. (1994) Character displacement, sexual dimorphism, and morphological variation among British and Irish mustelids. *Ecology*, **75**, 1063–1073.

Dayan T. & Simberloff D. (2005) Ecological and community-wide character displacement: The next generation. *Ecology Letters*, **8**, 875–894.

Dayan T., Simberloff D., Tchernov E., & Yom-Tov Y. (1990) Feline canines: community-wide character displacement among the small cats of Israel. *The American Naturalist*, **136**, 39–60.

Doube M., Wiktorowicz-Conroy A., Conroy A.W., Christiansen P., Hutchinson J.R., & Shefelbine S. (2009) Three-dimensional geometric analysis of felid limb bone allometry. *PloS one*, **4**, e4742.

Driscoll C.A., Yamaguchi N., Bar-Gal G.K., Roca A.L., Luo S., Macdonald D.W., & O'Brien S.J. (2009) Mitochondrial phylogeography illuminates the origin of the extinct caspian tiger and its relationship to the amur tiger. *PloS one*, **4**, e4125.

Ewer R.F. (1998) *The Carnivores.* Cornell University Press, New York.

Foster B.J. (1964) Evolution of mammals on islands. *Nature*, **202**, 234–235.

Gardner J.L., Peters A., Kearney M.R., Joseph L., & Heinsohn R. (2011) Declining body size: A third universal response to warming? *Trends in Ecology and Evolution*, **26**, 285–291.

Gause G.F. (1930) Studies on the Ecology of the Orthoptera. *Ecology*, **11**, 307–325.

Gay S.W. & Best T.L. (1996) Relationships betwen abiotic variables and geographic variation in skull of pumas (Puma concolor: Mammalia, Felidae) in North and South America. *Zoological Journal of Linnean Society*, **117**, 259–282.

Gohli J. & Voje K.L. (2016) An interspecific assessment of Bergmann's rule in 22 mammalian families. *BMC Evolutionary Biology*, **16**, 1–12.

Gonyea W.J. (1976) Functional implications of felid forelimb anatomy. *Acta Anatomica (Basel)*, **96**, 81–96.

Greenberg R., Cordero, Pedro J., Droege S., & Fleischer R.C. (1998) Morphological Adaptation with No Mitochondrial DNA Differentiation in the Coastal Plain Swamp Sparrow Author (s): Russell Greenberg , Pedro J . Cordero , Sam Droege and Robert C . Fleischer Published by : Oxford University Press Stable URL : https://www. *The Auk*, **115**, 706–712.

Guo Q., Liu Y., & Wieczorek J. (2008) Georeferencing locality descriptions and computing

associated uncertainty using a probabilistic approach. *International Journal of Geographical Information Science*, **22**, 1067–1090.

Hernández-Romero P.C., Guerrero J.A., & Valdespino C. (2015) Morphological variability of the cranium of Lontra longicaudis (Carnivora: Mustelidae): a morphometric and geographic analysis. *Zoological Studies*, **54**, 50.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., & Jarvis A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Ho C., Pennings S.C., & Carefoot T.H. (2010) Is Diet Quality an Overlooked Mechanism for Bergmann's Rule? *The American Naturalist*, **175**, 269–276.

Howell A.B. (1917) Pathologic Skulls of Captive Lions. *Journal of Mammalogy*, **6**, 163–168.

Jonsson B. & Jonsson N. (2001) Polymorphism and speciation in Arctic charr. *Journal of Fish Biology*, **58**, 605–638.

Khorozyan I. (2014) Morphological variation and sexual dimorphism of the common leopard (Panthera pardus) in the Middle East and their implications for species taxonomy and conservation. *Mammalian Biology - Zeitschrift für Säugetierkunde*, **79**, 398–405.

Kitchener A.C. (1999) Tiger Distribution, Phenotypic Variation and Conservation Issues. *Riding the Tiger. Tiger Conservation in Human-Dominated Landscapes* (ed. by J. Seidensticker, J. Christie, and P. Jackson), pp. 19–39. Cambridge University Press, Cambridge.

Kitchener A.C., Breitenmoser-Wuersten C., Eizirik E., Gentry A., Werdelin L., Wilting L., Wilting A., Yamaguchi N., Abramov A. V., Christiansen P., Driscoll C.A., Duckworth J.W., Johnson W., Luo S.-J., Meijaard E., O'Donoghue P., Sanderson J., Seymour K., Bruford M., Groves C., Hoffmann M., K. N., Timmons Z., & Tobe S. (2017) A revised taxonomy of the Felidae. The final report of the Cat Classification Task Force of the IUCN/SSC Cat Specialist Group. *Cat News Special Issue 11*, .

Kitchener A.C. & Dugmore A.J. (2000) Biogeographical change in the tiger , Panthera tigris. *Animal Conservation*, **3**, 113–124.

Kitchener A.C. & Yamaguchi N. (2010) What is a tiger? Biogeography, morphology, and taxonomy. *Tigers of the World* (ed. by R. Tilson and P. Nyhus), pp. 53–84. Academic Press, London.

Langerhans R.B., Layman C.A., Langerhans A.K., & Dewitt T.J. (2003) Habitat-associated morphological divergence in two Neotropical fish species. *Biological Journal of the Linnean Society*, **80**, 689–698.

Ledevin R. & Millien V. (2013) Congruent morphological and genetic differentiation as a signature of range expansion in a fragmented landscape. *Ecology and Evolution*, **3**, 4172–4182.

Liu Y.-C., Sun X., Driscoll C., Miquelle D.G., Xu X., Martelli P., Uphyrkina O., Smith J.L.D., O'Brien S.J., & Luo S.-J. (2018) Genome-Wide Evolutionary Analysis of Natural History and Adaptation in the World's Tigers. *Current Biology*, **28**, 3840–3849.e6.

Lorenzen E.D., Heller R., & Siegismund H.R. (2012) Comparative phylogeography of African savannah ungulates. *Molecular Ecology*, **21**, 3656–3670.

MacDonald D. & Loveridge A. (2010) *The biology and conservation of wild felids.* Oxford University Press, Oxford.

Mazak J.H. (2010) Geographical variation and phylogenetics of modern lions based on craniometric data. *Journal of Zoology*, **281**, 194–209.

Mazák J.H. (2010) Craniometric variation in the tiger (Panthera tigris): Implications for patterns of diversity, taxonomy and conservation. *Mammalian Biology - Zeitschrift für Säugetierkunde*, **75**, 45–68.

Mazák J.H. & Groves C.P. (2006) A taxonomic revision of the tigers (Panthera tigris) of Southeast Asia. *Mammalian Biology*, **71**, 268–287.

Meijaard E. (2004) Biogeographic history of the Javan leopard Panthera pardus based on a craniometric analysis. *Journal of Mammalogy*, **85**, 302–310.

Meiri S. & Dayan T. (2003) On the validity of Bergmann ' s rule. *Journal of Biogeography*, **30**, 331–351.

Meiri S., Dayan T., & Simberloff D. (2005) Area, isolation and body size evolution in insular carnivores. *Ecology Letters*, **8**, 1211–1217.

Metzger M.J., Bunce R.G.H., Jongman R.H.G., Sayre R., Trabucco A., & Zomer R. (2013) A high-resolution bioclimate map of the world: A unifying framework for global biodiversity research and monitoring. *Global Ecology and Biogeography*, **22**, 630–638.

Millien V. (2006) Morphological evolution is accelerated among island mammals. *PLoS Biology*, **4**, 1863–1868.

Mowat G. & Heard D.C. (2006) Major components of grizzly bear diet across North America. *Canadian Journal of Zoology*, **84**, 473–489.

Muñoz M.E. de S., De Giovanni R., de Siqueira M.F., Sutton T., Brewer P., Pereira R.S., Canhos D.A.L., & Canhos V.P. (2011) openModeller: A generic approach to species' potential distribution modelling. *GeoInformatica*, **15**, 111–135.

O'Hara R.B. (2005) The anarchist's guid to ecological theory. Or, we don't need no stinkin' laws. *Oikos*, **110**, 390–393.

O'Regan H.J. & Kitchener A.C. (2005) The effects of captivity on the morphology of captive, domesticated and feral mammals. *Mammal Review*, **35**, 215–230.

Pergams O.R.W. & Lawler J.J. (2009) Recent and widespread rapid morphological change in rodents. *PLoS ONE*, **4**, .

Phillimore A.B. & Owens I.P.F. (2006) Are subspecies useful in evolutionary and conservation biology? *Proceedings. Biological sciences / The Royal Society*, **273**, 1049–1053.

Platz S., Hertwig S.T., Jetschke G., Krüger M., & Fischer M.S. (2011) Comparative morphometric study of the Slovakian wildcat population (Felis silvestris silvestris): Evidence for a low rate of introgression? *Mammalian Biology*, **76**, 222–233.

Postma E. & Noordwijk A.J. Van (2005) Gene flow maintains a large genetic difference in clutch size at a small spatial scale. *Nature*, **433**, 65–68.

Randau M., Goswami A., Hutchinson J.R., Cuff A.R., & Pierce S.E. (2016) Cryptic complexity in felid vertebral evolution: shape differentiation and allometry of the axial skeleton. *Zoological Journal of the Linnean Society*, .

Shankaranarayanan P., Banerjee M., Kacker R.K., Aggarwal R.K., & Singh L. (1997) Genetic variation in Asiatic lions and Indian tigers. *Electrophoresis*, **18**, 1693–1700.

Sicuro F.L. & Oliveira L.F.B. (2011) Skull morphology and functionality of extant Felidae (Mammalia: Carnivora): A phylogenetic and evolutionary perspective. *Zoological Journal of the Linnean Society*, **161**, 414–462.

Smuts G.L., Anderson J.L., & Austin J.C. (1978) Age determination of the African lion (Panthera leo). *Journal of Zoology*, **185**, 115–146.

Sunquist M. & Sunquist F. (2002) *Wild Cats of the World.* University of Chicago Press, Chicago.

Team R.C. (2015) R: A language and environment for statistical computing. .

Teplitsky C. & Millien V. (2014) Climate warming and Bergmann's rule through time: Is there any evidence? *Evolutionary Applications*, **7**, 156–168.

Tomassini A., Colangelo P., Agnelli P., Jones G., & Russo D. (2014) Cranial size has increased over 133 years in a common bat, Pipistrellus kuhlii: A response to changing climate or urbanization? *Journal of Biogeography*, **41**, 944–953.

Torstrom S.M., Pangle K.L., & Swanson B.J. (2014) Shedding subspecies: The influence of genetics on reptile subspecies taxonomy. *Molecular Phylogenetics and Evolution*, **76**,

134–143.

Viguier B. (2002) Is the morphological disparity of lemur skulls (Primates) controlled by phylogeny and/or environmental constraints? *Biological Journal of the Linnean Society*, 577–590.

Wieczorek J., Guo Q., & Hijmans R. (2004) The point-radius method for georeferencing locality descriptions and calculating associated uncertainty. *International Journal of Geographical Information Science*, **18**, 745–767.

Wilting A., Courtiol A., Christiansen P., Niedballa J., Scharf A.K., Orlando L., Balkenhol N., Hofer H., Kramer-Schadt S., Fickel J., & Kitchener A.C. (2015) Planning tiger recovery: Understanding intraspecific variation for effective conservation. *Science Advances*, **1**, 1–13.

Winker K. (2010) Subspecies Represent Geographically Partitioned Variation, A Gold Mine of Evolutionary Biology, and a Challenge for Conservation. *Ornithological Monographs*, 6–23.

Yamaguchi N., Driscoll C.A., Werdelin L., Abramov A. V, Cuisin J., Fernholm B., Hiermeier M., Hills D., Hunter L., Johansson U.S., Kascheev V., Krohmann K., Martin T., Pavlinov I.Y., Renoud F., Tomsett L., Mije S. Van Der, Groves C., Kitchener A.C., Nijman V., & Macdonald D.W. (2013) Locating Specimens of Extinct Tiger (Panthera tigris) Subspecies: Javan Tiger (P.T. sondaica), Balinese Tiger (P.T. balica), and Caspian Tiger (P.T. virgata), Including Previously Unpublished Specimens. *The Mammal Society of Japan*, **38**, 187–198.

Zomer R.J., Bossio D.A., Trabucco A., Yuanjie L., Gupta D.C., & Singh V.P. (2007) Trees and Water: Smallholder agroforestry on irrigated lands in Northern India. Columbo, Sri Lanka: International Water Management Institute. .

Zomer R.J., Trabucco A., Bossio D.A., & Verchot L. V. (2008) Climate change mitigation: A spatial analysis of global land suitability for clean development mechanism afforestation and reforestation. *Agriculture, Ecosystems and Environment*, **126**, 67–80.

Chapter 7: Synthesis and Overall Conclusions

Biogeographical Modelling

The original biogeographical modelling presented here has shed light upon the expanding and contracting ranges of the lion and the tiger at key times since the Last Glacial Maximum, and provides a proxy for possible distributional change during the changing glacial/interglacial, and pluvial/interpluvial conditions of the Pleistocene. The results of *Chapter 3* show that without the impacts of anthropogenic pressure and the resulting habitat and population fragmentation, the present day, interglacial range of the tiger would likely be contiguous through the Indian Subcontinent, Indochina, China and the Russian Far East. Wetter conditions during the mid-Holocene would have increased connectivity to the Caspian region enabling tigers to expand westward from the Russian Far East. The Last Glacial Maximum, and previous glacial conditions by proxy, would have caused a northern contraction in the range of the tiger, yet allowed expansion and greater connectivity between tigers within the exposed Sunda Shelf, which provided favourable bioclimatic conditions to the tiger. The continuous modelled distribution of the tiger in mainland Asia supports the notion of unimpeded gene flow between all populations of the tiger throughout the late Pleistocene and Holocene, with only recent sea level rise (post LGM) separating the Sunda Island tigers from one another and from the mainland, and anthropogenic disturbance separating Continental populations from one another. The modelling results presented allow the discussion of further environmental influences which may have constrained the tiger. The super-volcanic eruption of Mt. Toba likely reduced tiger populations to a core southern range ~73ka, which is supported by genetic evidence of a recent common ancestor around this time.

Chapter 4 builds upon the Maximum Entropy modelling approach of *Chapter 3*, and provides a novel bioclimatic perspective by describing preferred bioclimatic conditions for the lion in terms of Global Environmental Strata and Zones. The results show that there has been a general reduction in lion range from the LGM through the mid-Holocene to the present day. Favourable habitat within sub-Saharan Africa has largely remained contiguous through different bioclimatic conditions, and so the long term phylogenetic divergence between

populations has likely come from non-climatic barriers such as large rivers, lakes and mountains. Similarly there is no clear bioclimatic explanation, at the continental scale of these models, for lion expansion through the Near-East into the Indian Subcontinent. Due to climate model inconsistencies with alternative sources of data in the Sahara, conclusions regarding past lion distributions in North Africa and the Near East are as much based on proxy data as through the modelled results presented. This is because there is strong evidence supporting a Green Sahara episode at the time of the Mid-Holocene, but a current lack of suitable continuous bioclimatic data for this time period to inform species distribution models. As a result there is a potential for major range changes in northern Africa and the Near East from the Last Glacial Maximum to the present.

The results presented in *Chapters 3* and *4* show a pattern of tiger ranges benefitting from warmer and wetter interglacial conditions (except where land masses are obscured by sea level rise), whereas the preferred bioclimatic conditions of the lion have reduced since the Last Glacial Maximum to present interglacial conditions (despite the temporary increase in favourable habitat in North Africa and Arabia likely afforded by the greening of the Sahara during the mid-Holocene). This is in part due to each species preferred niche in that the tigers preferred range involves greater forest cover, whereas lions are associated with more open savannah and grassland habitats. The transition to warmer and wetter conditions during interglacial periods has led to a northward expansion of forest cover for the tiger, yet for the lion increased equatorial forests have decreased their potential range. The patterns of expansion and contraction also differ due to very different climate forcing influences between Asia and Africa, particularly due to the pluvial/interpluvial cycles from monsoonal circulation variability, which have dictated lion distributions through North Africa and the Near East.

The patterns of expansion and contraction of the potential ranges of each species is of particular interest in the Near East and Indian Subcontinent, where both species have persisted into historic and modern times. The modelling of *Chapter 3* suggests that the tiger could have maintained a persistent presence within the Indian Subcontinent through glacial and interglacial cycles, and that tigers in the Caspian region through to Northern Iran are likely to be relatively recent Holocene migrants, who exploited more favourable conditions extending from the Russian Far East after the end of the Last Glacial Maximum. Conversely, whilst lions have persisted in Western India into modern times, the modelling presented in

Chapter 4 shows that much larger expanses of the Indian Subcontinent were preferable to the modern lion during glacial periods. Although genetic evidence suggests that the lion has moved out of Africa in relatively recent waves of migration, it is possible that the lion has restricted the range of the tiger within the Indian Subcontinent during periods of the Late Pleistocene. Future modelling of the changing range of the lion and the tiger within the Near East and Indian Subcontinent during the Late Pleistocene would greatly benefit from improvements to the climatic model data within North Africa and the Arabian Peninsula, the shortcomings of which are discussed in *Chapter 5*.

The modelling presented in *Chapters 3* and *4* shows the broad applicability of coarse scale biogeographical modelling, yet inconsistencies exist between the models presented and existing phylogenetic analysis, historical data, and climate proxies. A fundamental issue that arises for both the lion and the tiger is that the potential for narrow yet significant biogeographical barriers such as rivers is poorly accounted for by course bioclimatic data. This issue is illustrated by the linear tugai habitat in the Caspian region which is not identified in models for the tiger and yet has provided favourable strips of habitat into modern times which significantly extended the tigers' range. Likewise similar potential linear pathways of favourable habitat are not identified within the Near East for the lion. Narrow bands of contrasting habitats can have both negative (barrier) as well as positive (pathway) effects. The likely riparian barriers proposed as separating clades in the lion do not appear within areas of preferable climatic suitability in the modelled results, and in a similar way, the possible negative influence of major rivers within Sundaland on tiger dispersal may have been similarly obscured by model resolution. The influence of rivers as barriers to, or conduits for dispersal is not easily modelled, given the ability for both the lion and especially the tiger to cross large bodies of water. The influence of rivers may also vary through time which further complicates our understanding their influence, as seasonal change may greatly affect their efficacy and persistence as boundaries or as dispersal corridors.

Whilst the lion and tiger are large, mobile generalists, which are well suited to modelling using coarse bioclimatic data, this research highlights the need to consider the influence of linear geographic features as conduits or barriers to dispersal, either explicitly, or within the context of proxy evidence such as genetic differentiation. The correlative models presented in this thesis provide a novel basis for a wider discussion of stochastic events, biological

interactions and rising anthropogenic influences, which are expanded upon from alternate sources of data through the Late Pleistocene and Holocene.

The modelling of *Chapters 3* and *4* provides geographical context to the phylogenetic patterns found within modern populations of the lion and tiger, highlighting where broad climatic tolerance does (and does not) explain long term divergence or contiguity between populations.

Skull Morphology

Chapter 5 examines the extent to which bone is a phenotypically plastic tissue, and how skull morphology can change given vastly different environmental conditions and masticatory stresses posed by a captive lifestyle. The results show that captivity can lead to differentiation in skull characteristics between captive and wild specimens, which has meaningful implications for both the managers of zoological collections, and also to existing and future assessments of morphological differentiation of populations in the wild. *Chapter 5* therefore acts as a necessary precursor to the analysis of wild specimens in *Chapter 6*, by highlighting the measurements within the utilised dataset which are particularly susceptible to changes in life history, to better understand the nature of morphological differentiation between wild populations. This research highlights that skulls are generally wider in captive individuals, whereas mandible heights are larger in wild specimens, and proposes these changes are due to an increase in the forces routinely applied to the skull in wild specimens through feeding upon natural prey in their entirety - flesh, bone and connective tissue.

Building upon *Chapter 5*, morphological differentiation in wild specimens is considered in *Chapter 6* in relation to phenotypic plasticity, short and long term evolutionary processes, and the biogeographical context of variation. The relationship between long term evolutionary divergence and morphological disparity is not always apparent. Multiple scales of variation, from phenotypic plasticity, to evolutionary selection and genetic fixation have likely played a role in the differentiation of specimens of both the lion and the tiger. The clinal variation in size in both the lion and the tiger, which broadly corresponds to latitudinal change, is most likely a micro-evolutionary response to prevailing environmental conditions,

although the exact cause of size selection is obscured by multiple broad biogeographic drivers which could dictate changes to body size. Shape differentiation of the Amur population of the tiger and of the Asiatic population of the lion corresponds to measurements which are shown to vary due to phenotypic plasticity, with limited differentiation in variables likely to account for evolutionary divergence. The vastly different climatic conditions, and prey composition and density has likely driven morphological disparity in these populations. The lack of samples between each respective population has emphasized their differences, which would likely show clinal shape change given more lion specimens from the Near East, and tiger specimens from mainland China. Shape differentiation in the tiger between the Sunda Islands likely represents a combination of the random fixation of genes due to population isolation, and allometric scaling due to differentiation in skull size.

The morphological analysis presented here shows the importance of considering the mechanisms of differentiation at multiple scales, and the need to identify morphological changes driven by life histories and those determined by inheritance. A prior understanding of patterns of phenotypic plasticity of individual specimens have profound implications for the study of morphological variation amongst wild populations, as groups divided by morphological characteristics may only represent differences in life histories. Whilst the results of *Chapter 5* feed into the understanding of wild morphological variation outlined in *Chapter 6*, an understanding of how parts of the skull vary in the wild is useful in understanding the possible pathological nature of certain conditions found in captivity such as cranial volume and foramen magnum stenosis in the lion.

Linking Biogeographical Analysis to Morphological Analysis

Chapters 3-5 provide crucial context to the patterns of wild variation found in *Chapter 6*. The modelling presented explores the possible evolutionary drivers of change to populations of the lion and tiger, either through long term vicariance due to bioclimatic conditions and biogeographic boundaries, or from the disparity between potentially suitable habitat and recently fragmented populations due to anthropogenic pressure. *Chapters 3* and *4* highlight that both the Amur tiger and Asian lion are founding and relict populations respectively, outside of the core range of each species, and that anthropogenic disturbance has further isolated these populations for hundreds, to thousands of years. In isolation this information

would provide a basis for explaining the differentiation in cranial shape of these groups from other lion and tiger populations, however the findings of *Chapter 5* show that there is likely a strong plastic component to skull differentiation in these populations. The differentiation in skull shape between the Sunda Island tigers is supported by the findings of *Chapter 4* which shows these populations have been increasingly separated from the mainland and one another, since the early Holocene as sea levels rose. The lack of morphological differentiation between lions and tigers which have occupied contiguous suitable modelled habitat within mainland Africa and Asia respectively, despite long term genetic divisions, shows that morphology is likely a result of environmental conditions, which are similar between neighbouring populations, or that even low level gene flow between populations has prevented step changes in variation between these groups.

Implications for Taxonomy and Conservation

The research presented here establishes a baseline of biogeographical understanding of both the present potential range of the lion and tiger without the influence of anthropogenic dissociation, and of the nature of contiguity and separation of populations through the Late Pleistocene and Holocene. The results of *Chapter 6*, which take into account the evolutionary histories and life histories of each species outlined within this thesis, highlights the origin of morphological differentiation of current populations.

The results presented here suggest that the Amur tiger has likely been dissociated from other Continental tigers from ~8ka, but morphological variation is predominantly caused by phenotypic plasticity due to vastly different life histories of this population. A lack of step changes in morphology (beyond those explained by phenotypic plasticity), and contiguous habitat conditions within mainland Asia do not support long term population vicariance between Continental tigers. The Sunda Island tigers show distinct morphological characteristics from one another and from the mainland, likely due to a combination of their separation through sea level rise following the Last Glacial Maximum but also to recent size differentiation due to prevailing environmental conditions and insular dwarfism. These findings are concordant with a two subspecies approach for the tiger, which recognises the Amur population as a separate management unit, but not a distinct subspecies, and the Sunda

Island tiger as distinct subspecies from Continental tiger (Kitchener & Yamaguchi, 2010; Wilting et al., 2015).

The lack of morphological differentiation between lions within Africa, and broadly contiguous environmental suitability between populations is discordant with identified evolutionary differentiation within the continent. Whilst the modelling presented does not explicitly account for significant narrow biogeographic barriers such as rivers and lakes, the continuous and similar nature of suitable environmental conditions presented likely accounts for the lack of morphological differentiation between neighbouring populations as differing selective pressures are low. Morphological separation of Asian lions, which are genetically similar to other North African lions despite their geographic isolation, is largely accounted for by plastic changes to the skull caused by differences in life history. These findings presented here provide new information for in-situ and ex-situ conservation management of increasingly fragmented wild populations.

Recommendations for Future Research

The results presented in this thesis highlight both the potential of and problems with coarse scale bio-geographical models, all of which could be addressed in future research. There is a need to investigate the applicability of integrating palaeo-river data into species distribution models when questions of long term population vicariance and connectivity are of importance. This will need to be informed by a better understanding of these features as barriers or facilitators of movement. *Chapter 4* highlights the critical importance of the palaeo-climatic models which underpin the species distribution models presented. Further modelling of the potential interactions between preferred lion and tiger habitat in the Near East and the Indian Subcontinent, where their ranges have historically overlapped, can only be completed for past climatic conditions when palaeo-climatic data for the Near East is in agreement with available proxy data. The assessment of the extent of phenotypic plasticity in the lion and tiger prior to the assessment of wild morphological variation has had large implications for the understanding of taxonomic differentiation in each species. Further investigation of this method with other species may provide further evidence of the importance of this two-step approach.

References

Kitchener A.C. & Yamaguchi N. (2010) What is a tiger? Biogeography, morphology, and taxonomy. *Tigers of the World* (ed. by R. Tilson and P. Nyhus), pp. 53–84. Academic Press, London.

Wilting A., Courtiol A., Christiansen P., Niedballa J., Scharf A.K., Orlando L., Balkenhol N., Hofer H., Kramer-Schadt S., Fickel J., & Kitchener A.C. (2015) Planning tiger recovery: Understanding intraspecific variation for effective conservation. *Science Advances*, **1**, 1–13.

Appendix 1:

Chapter 3 as published in Diversity and Distributions, 2016

Diversity and Distributions, (Diversity Distrib.) (2016) 22, 1199–1211

BIODIVERSITY RESEARCH

Predicted Pleistocene–Holocene range shifts of the tiger (*Panthera tigris*)

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ABSTRACT

Aim In this article, we modelled the potential range shifts of tiger (*Panthera tigris*) populations over the Late Pleistocene and Holocene, to provide new insights into the evolutionary history and interconnectivity between populations of this endangered species.

Location Asia.

Methods We used an ecological niche approach and applied a maximum entropy (MAXENT) framework to model potential distributions of tigers. Bioclimatic conditions for the present day and mid-Holocene, and for the Last Glacial Maximum (LGM), were used to represent interglacial and glacial conditions of the Late Pleistocene, respectively.

Results Our results show that the maximum potential tiger range during modern climates (without human impacts) would be continuous from the Indian subcontinent to north-east Siberia. During the LGM, distributions are predicted to have contracted to southern China, India and Southeast Asia and remained largely contiguous. A potential distribution gap between Peninsular Malaya and Sumatra could have effectively separated tigers on the Sunda Islands from those in continental Asia during interglacials.

Main conclusions The continuous modelled distribution of tigers in mainland Asia supports the idea of mainly unimpeded gene flow between all populations throughout the Late Pleistocene and Holocene. Thus, our data support a pragmatic approach to tiger conservation management, especially of mainland populations, as it is likely that only recent anthropogenic changes caused separation of these populations. In contrast, Sunda tigers are likely to have separated and differentiated following the Last Glacial Maximum and thus warrant separate management.

Keywords
ecological niche model, evolutionary history, maximum entropy, *Panthera tigris*, Pleistocene distribution, Toba.

INTRODUCTION

Tigers are endangered and occupy only a small fraction of their historical range (Walston *et al.*, 2010). The changing isolation or interconnectivity of tiger populations has wide-ranging implications for both *in situ* and *ex situ* conservation efforts, as conservationists currently aim to preserve what are assumed to be genetically distinct populations whilst attempting to strengthen the numbers of captive and wild tiger populations. This article presents a new, geographically based assessment of changes in tiger distribution during the Late Pleistocene and Holocene, to understand the degree of natural connectivity between separate tiger populations and thus inform current conservation efforts.

With a large subcontinental-scale geographical range, it is not surprising that tigers display morphological variation in response to regional differences in climate and habitat. This differentiation is reflected in the recognition of up to nine subspecies of tiger (Goodrich *et al.*, 2015; but see Wilting *et al.*, 2015; Fig. 1a), and these are the focus of current

DOI: 10.1111/ddi.12484

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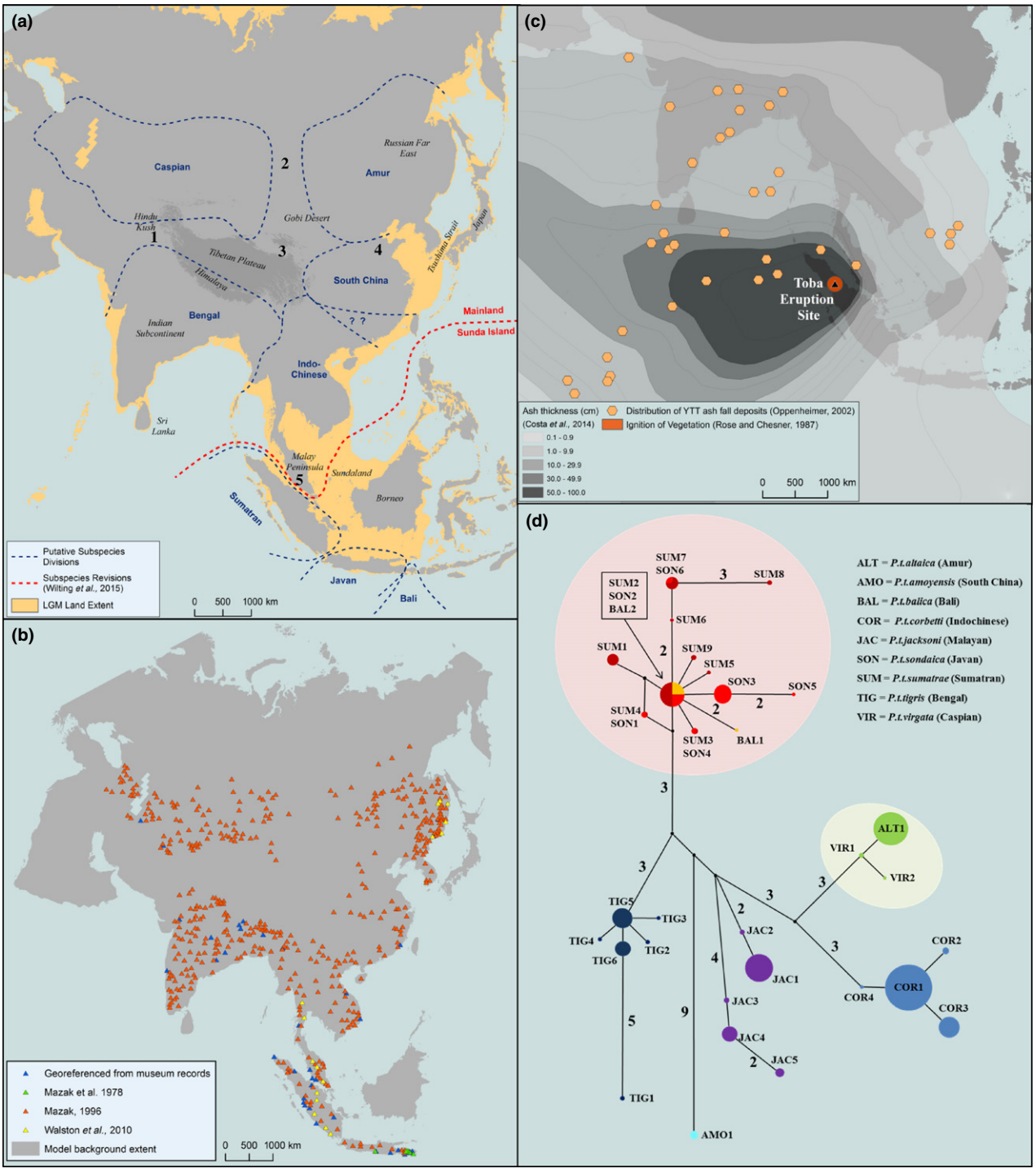

Figure 1 (a) Established subspecies divisions (Mazák, 1996) and revised divisions (Wilting *et al.*, 2015). Potential corridors for tiger dispersal between populations are (1) Southern/Himalayan Corridor, (2) Northern Corridor and (3) Gansu/Silk Road Corridor (Driscoll *et al.*, 2009). (4) Huanghe river catchment and site of human agricultural civilization ca. 8 ka, (5) Additional recognition of a Malayan tiger (*P.t. jacksoni*) (Luo *et al.*, 2004). (b) Tiger locality data, coloured by data source, with the background extent used for modelling. (c) Effects of the 73 ka Toba super-eruption. The initial eruption effects, cooler temperatures and drought induced by the ejecta, followed by rapidly decreasing tree cover in the decades following the Toba eruption, might have been responsible for genetic bottlenecks in Southeast Asia (Robock *et al.*, 2009; Williams *et al.*, 2009; Wilting *et al.*, 2011, 2012). (d) Haplotype network of nine putative subspecies (Wilting *et al.*, 2015) – circle size is proportional to haplotype frequency; lines represent a single mutational step, or multiple steps as indicated by numbers.

conservation efforts. However, the scientific integrity of so many subspecies has been questioned (e.g. Herrington, 1987; Kitchener, 1999; Kitchener & Yamaguchi, 2010; Wilting *et al.*, 2015). It is possible these apparent patterns of variation are the result of genetic drift due to increasing recent fragmentation of tiger populations (Luo *et al.*, 2004; Mondol *et al.*, 2013), but lack conservation and evolutionary significance (Wilting *et al.*, 2015). Although the fossil record of the tiger stretches back more than 2.5 million years (Mazák *et al.*, 2011), studies of mitochondrial DNA have estimated that the most recent common ancestor for today's tiger populations existed 72–108 ka (Luo *et al.*, 2004; Wilting *et al.*, 2015). As a result, environmental changes in the Late Pleistocene and Early Holocene are most relevant when trying to understand differentiation of today's local tiger populations. For example, Sunquist (1981) suggested that the initial adaptive radiation of modern tigers may have occurred during the Pleistocene glaciations when Southeast Asian climates were drier (Whitmore, 1984), sea levels were lower, the Sunda Islands were linked by land to the Asian mainland, and insular and mainland populations could mix. Subsequent sea-level rises isolated tigers of the Sunda Islands from mainland tigers and each other, possibly leading to local differentiation. In addition to range changes driven by glacial cycles, the super-eruption of Toba ca. 73 ka in northern Sumatra (Fig. 1c) may have played a key role in the evolutionary history of tigers through extensive habitat loss. This eruption produced around 2500–3000 km^3 of dense rock-equivalent pyroclastic ejecta (Rose & Chesner, 1987) with associated Younger Toba tuff (YTT) deposits found in cm–scale thicknesses across the Indian subcontinent (Acharyya & Basu, 1993; Shane *et al.*, 1995), and at a thickness of more than four metres close to the Toba caldera (Oppenheimer, 2002). The outflow of m-scale deposits of YTT could have resulted in the ignition of vegetation across 30,000 km^2 around the caldera (Oppenheimer, 2002).

The evolutionary history of modern tiger populations has been assessed phylogenetically based on geographically referenced specimens (or at least specimens from known putative subspecies) and the analysis of mitochondrial DNA (Cracraft *et al.*, 1998; Luo *et al.*, 2004; Driscoll *et al.*, 2009; Wilting *et al.*, 2015; Xue *et al.*, 2015), and/or nuclear microsatellite genotypes (Luo *et al.*, 2004). A haplotype network was constructed by Wilting *et al.* (2015) (Fig. 1d). Phylogenetic approaches to understanding tiger populations are constrained because they are based on limited sampling of already fragmented populations (Luo *et al.*, 2004). Additionally, current phylogeographical studies, for example Luo *et al.* (2004), Driscoll *et al.* (2009) have only a limited spatial component (Waltari *et al.*, 2007; Kozak *et al.*, 2008). To help understand phylogeographical patterns seen today, there is a need for a geographically explicit understanding of the expansion and contraction of tiger ranges during glacial/interglacial cycles.

The understanding of the evolutionary history of tigers is of great importance for the successful conservation of this highly threatened species. Recognizing too few differentiated populations could see the loss of important evolutionary diversity, but recognizing too many would lead to a waste of resources and may compromise conservation efforts because some remaining populations are too small to survive (Bay *et al.*, 2014). In contrast to some molecular studies which support high differentiation among mainland tiger populations (Luo *et al.*, 2014; but see Wilting *et al.*, 2015), Kitchener & Dugmore (2000) used a biogeographical approach and their results showed a considerable contiguity between mainland tiger populations. However, 16 years have elapsed since that research was undertaken, and there is scope for radical improvement using new and improved modelling techniques and data. Here, we examine the global distribution of the tiger using a maximum entropy (MAXENT) niche modelling approach to reconstruct the current range of the tiger based on bioclimatic variables, assuming no human impacts. We further modelled the global ranges at the LGM and mid-Holocene to represent the maximum range of bioclimatic variation to impact tiger distribution and to assess potential differentiation by isolation since modern populations originated ca. 100 ka.

METHODS

Tigers need access to water, and they require dense vegetation cover of sufficient area to support large ungulates and to hunt their prey successfully (Nowell & Jackson, 1996). Therefore, the modelling approach used here assumes that tigers are generalists, operating within certain kinds of vegetation cover, and prey size and abundances that are dictated by climate. Climate is a key factor in defining ecological niches and the geographical distribution of species at continental scales (Geffen *et al.*, 2004; Varela *et al.*, 2010) and has been used to model megafaunal ranges (Varela *et al.*, 2010; Lorenzen *et al.*, 2011). Niche models, comparing recent and fossil locality records, have shown that mammal species have tracked consistent climate profiles since the LGM (Martínez-Meyer *et al.*, 2004) and therefore climate change may be used to infer differences in mammal range as it is unlikely that a generalist, adaptable carnivore such as the tiger has shifted climatic niche through the Late Pleistocene.

Tiger locality records were taken from Mazák (1996) (448 locations), Walston *et al.* (2010) (16 locations), Mazák *et al.* (1978) (7 locations) and 40 localities georeferenced from European museum specimens (Fig. 1b). The records cover the geographical extent of the known distribution of tigers in modern times and likely represent the potential climatic niche of the tiger, but it cannot be discounted that human extirpation of the tiger has reduced the potential niche space represented by our records. Nine contemporary WorldClim bioclimatic variables (Hijmans *et al.*, 2005), in combination with the tiger localities, were used to produce a global habitat suitability model for the tiger using MAXENT.

Of *presence only* modelling techniques, MAXENT models have been shown to perform as well or better than other existing approaches (Elith *et al.*, 2006; Hernandez *et al.*,

2006; Phillips *et al.*, 2006). Hernandez *et al.* (2006) found that MAXENT performed well regardless of the number of species records or the geographical extent of records, compared to Mahalanobis Typicalities and Random Forests methods. MAXENT software (Phillips *et al.*, 2006) was used for modelling. In addition to MAXENT, a boosted regression tree (BRT) model was created for comparison (see Appendix S1 in Supporting Information). Models were created using WorldClim variables at a 2.5-arc-minute resolution (<5 km), which is between one and two orders of magnitude less than the scale of individual tiger ranges. This resolution was chosen to show potential habitat suitability through narrow geographical corridors such as through valleys or mainland connections to peninsulas which may affect gene flow between tiger populations. Elevation was not included as a separate independent variable because it is accounted for in the bioclimatic datasets. In contrast to Kitchener & Dugmore (2000), vegetation data, such as Biome 4 (Kaplan *et al.*, 2003), have not been included in the model, because they can decrease performance in predicting presences compared to models based only on climatic variables (Martínez-Meyer *et al.*, 2004). A comparison with Biome 4 data was used as a post-modelling measure of validity, by checking that modelled high habitat suitability does not occur in highly unfavourable habitats (Desert and Tundra), as they are unequivocally not recognized as tiger habitat (Nowell & Jackson, 1996).

Highly correlated variables were removed from the modelling process to reduce over-parameterization and loss of predictive power (Buermann *et al.*, 2008; Garcia-Porta *et al.*, 2012). The removal of highly correlated variables ($r \geq 0.8$), calculated using ArcGIS Band Collection Statistics (ARCMAP v10.1), from the 2.5-arc-minute WorldClim bioclimatic dataset reduced the number of variables from 19 to nine (annual mean temperature, mean diurnal range, isothermality, mean temperature of the wettest quarter, precipitation seasonality, precipitation of the wettest quarter, precipitation of the driest quarter, precipitation of the warmest quarter, precipitation of the coldest quarter). Where one or more variables were highly correlated, the one deemed most important was selected using indicators, such as performance in a jackknife test (Pearson *et al.*, 2006).

MAXENT models were run under settings so as to fit a Poisson point-process model –'noremoveduplicatepresencerecords' and 'noaddsamplestobackground', with final projections displaying MAXENT raw output (Renner *et al.*, 2015). MAXENT models were evaluated under a range of settings to assess their effects on model performance – see Appendix S1 for a full analysis of parameter selection. Final MAXENT models were run using a regularization multiplier (RM) of 2, and 100,000 background points. Ideally, models would be evaluated with independent observation data; however, these data are not available. Instead, cross-validation was performed using replicates in MAXENT to assess each model. Ten runs of each model were performed, and the mean area under the receiver operating characteristic curve (AUC) was used as a measure of overall performance (Phillips & Dudík, 2008). Additionally, spatially independent cross-validation was performed using the checkerboard2 method implemented via the ENMeval package (Muscarella *et al.*, 2014) in R (R Core Team, 2015) to reduce the potential effects of spatially autocorrelated localities, which may inflate the AUC (Veloz, 2009). Checkerboard2 was implemented with coarse grids 200 and 400 times the resolution of the bioclimatic variables (~450 km^2 and ~900 km^2 at the equator, respectively) to ensure considerable geographical separation of training and testing localities. Models were run over a geographical extent covering the known and potential distribution of the tiger (82° N to 10° S, 20° W to 144° E). Africa and islands not connected to continental Asia during glacial conditions, such as those east of the Wallace's Line, were removed from the extent before modelling.

Following assessment, the model was fitted using all localities and projected to the LGM at 21 ka and mid-Holocene at 6ka, using WorldClim data derived from the MIROC-ESM, CCSM-4 and MPI-ESM-P coupled general circulation models (GCMs) (Hijmans *et al.*, 2005) based on CMIP5 (Taylor *et al.*, 2012) data. Model clamping was implemented to restrict variables to the range of values encountered during training. An ensemble projection was created for the mid-Holocene and LGM based upon the mean raw value of the projected models from the three coupled GCM datasets for each time period. The individual projections and range of the projection values can be viewed in Appendix S1. The present/mid-Holocene and LGM climates provide bounding values of the climatic fluctuations of the Pleistocene and thus likely represent environmental changes that influenced tiger distribution and potential range shifts. Whilst the present and mid-Holocene both represent interglacial climatic conditions, with both periods exhibiting similar global annual mean temperature and precipitation, the mid-Holocene is associated with an enhanced seasonal cycle in the Northern Hemisphere and wetter conditions within eastern Asia (Braconnot *et al.*, 2007; Tao *et al.*, 2010), which may have affected tiger distributions.

Georeferenced fossil records of the tiger from the Late Pleistocene (Kitchener & Yamaguchi, 2010) are presented on the relevant glacial/interglacial habitat suitability map based on their estimated age (Fig. 2a,b). The Late Pleistocene distribution of the ancestors of the modern lion (*Panthera leo*) and of the Eurasian cave lion (*Panthera leo spelaea*) (Barnett *et al.*, 2009) have been superimposed onto modelled tiger distributions [Fig. 3(6)], because where open habitat is dominant, it is expected that the group-living modern lion, and likely group-living Eurasian cave lion (Yamaguchi *et al.*, 2004) would out-compete tigers and prevent their dispersal. Estimates of ashfall from the very large scale volcanic eruption of Toba ca. 73 ka (Costa *et al.*, 2014) have also been added post-modelling [Fig. 3(1)] to assess their potential impacts on tiger distributions. The minimum values of the raw output between present day, mid-Holocene and LGM projections are presented in Fig. 3[~73ka(+1k)], to represent

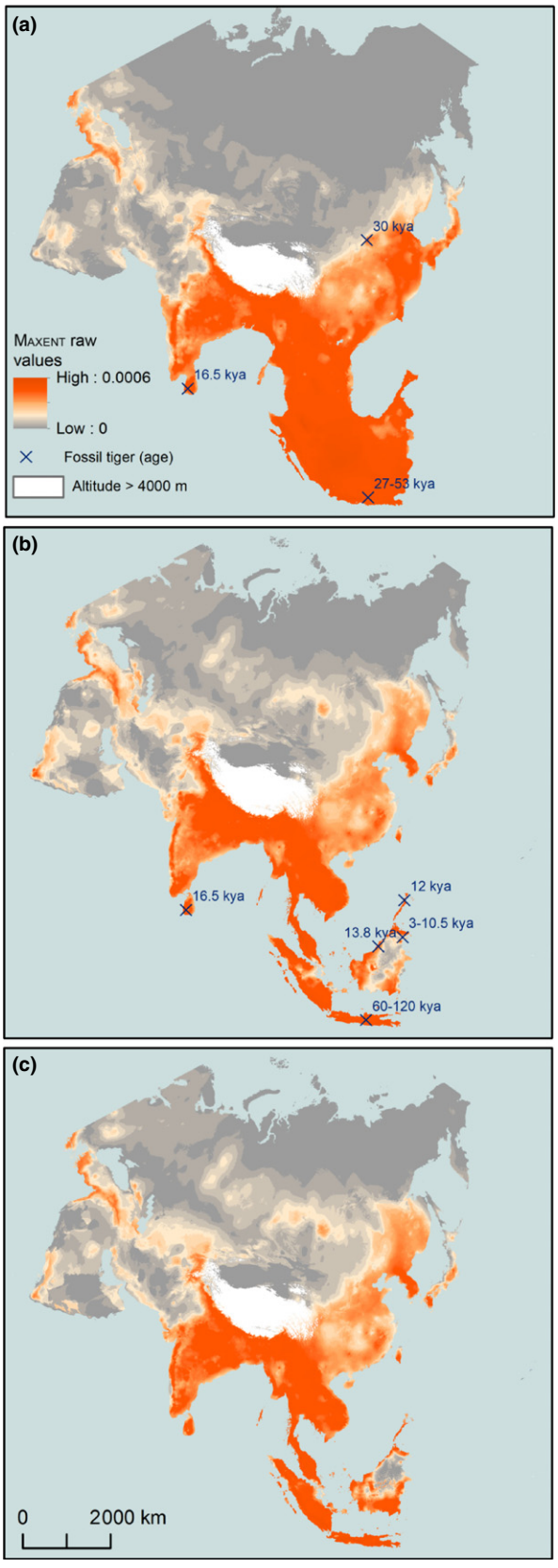

Figure 2 Raw MAXENT output displaying modelled tiger habitat suitability based on nine bioclimatic variables, projected using an Asia Lambert Conformal Conic Projection, for the LGM (a), mid-Holocene (b) and present day (c). Elevations > 4000 m (highlighted in white) have been included as a possible barrier to dispersal. Fossil tiger localities are laid over glacial (a) and interglacial (b) projections in accordance with the fossil age (note 16.5 kya from Sri Lanka is included in both scenarios due to the transitionary time period following the LGM).

a rapid shift from interglacial to glacial conditions during the 1000 years following the Toba eruption (Rampino & Self, 1992; Harris, 2008).

RESULTS

The tiger distribution model run under final parameters with cross-validation produced a mean AUC of 0.843, representing the probability of a randomly chosen presence locality being ranked above a random background point (Phillips *et al.*, 2006). The same parameters, but run with spatially independent cross-validation, gave a mean AUC of 0.780. Under a point-process framework, the MAXENT raw projection is viewed as the intensity of potential locality reportings within a given area (Renner *et al.*, 2015), which for our projected models can be interpreted in terms of habitat suitability. Model sensitivity is further discussed in Appendix S1 – conclusions regarding tiger distributional changes from our chosen MAXENT model are consistent with MAXENT models created under a range of parameters, and with a simply implemented BRT model.

The fossil record of tigers corresponds well with predicted habitat suitability maps for glacial/interglacial conditions (Fig. 1a,b), although this evaluation method is limited by the scarcity of dated archaeological finds. A comparison of model projections with unfavourable vegetation provides support for past outputs, as strong modelled habitat suitability has not occurred in biomes deemed unfavourable (Appendix S1). Modelling the likely ranges for present-day climate (Fig. 2a) indicates a potentially contiguous tiger distribution from southern India to the Amur region, and presence throughout the Malay Peninsula, Sumatra, Java and Bali. The present model suggests that tigers in the Caspian region existed within suboptimal habitat, and likely connected through corridors of favourable habitat to tiger populations in northern India, and the Amur region. The core areas of tiger habitat, defined as those suitable at present, mid-Holocene and during the LGM (Fig. 1a–c), exist within the Indian subcontinent and Southeast Asia.

DISCUSSION

The present-day model corresponds well with the Habitat Topography Precipitation model of present tiger distribution (Kitchener & Dugmore, 2000) and historic tiger distributions during the Holocene (Dinerstein *et al.*, 2006). However, the present model predicts low habitat suitability for central and northern Borneo, whereas past studies have modelled this as suitable habitat (Kitchener & Dugmore, 2000). One implication is that simple associations with forest cover, annual

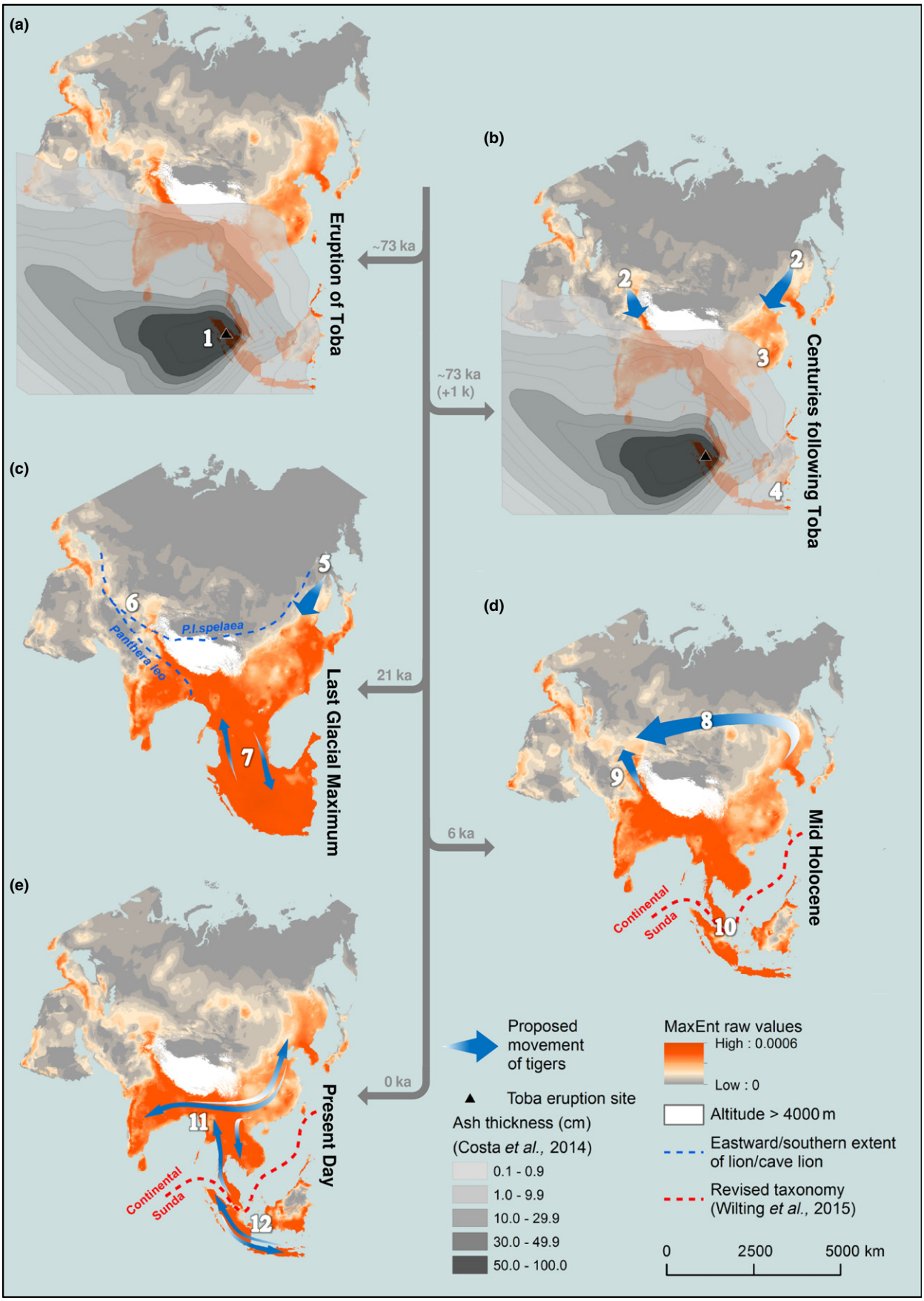
(a)
Eruption of Toba
1
~73 ka
(b)
Centuries following Toba
2
2
3
4
~73 ka
(+1 k)
(c)
Last Glacial Maximum
5
6
P.l.spelaea
Panthera leo
7
21 ka
(d)
Mid Holocene
8
9
Continental
Sunda
10
6 ka
(e)
Present Day
11
Continental
Sunda
12
0 ka
Proposed movement of tigers
Toba eruption site
Ash thickness (cm)
(Costa *et al.*, 2014)
0.1 - 0.9
1.0 - 9.9
10.0 - 29.9
30.0 - 49.9
50.0 - 100.0
MaxEnt raw values
High : 0.0006
Low : 0
Altitude > 4000 m
Eastward/southern extent of lion/cave lion
Revised taxonomy (Wilting *et al.*, 2015)
0
2500
5000 km

Figure 3 Proposed range shifts of the tiger since the ~73 ka eruption of Toba (a) (1). Immediate impacts (1), and millennial scale cooling (b), likely reduced the northern range (2), leading to a probable refugium (3), associated with the genetic bottleneck of the tiger. Suitable (but fragmented) habitats (4) were unlikely to have harboured the tiger during this time due to a single source of molecular variation. The northern range of the tiger would have been suppressed by unfavourable conditions at the LGM (c) (5), and Caspian/western Indian marginal habitat may have been impacted by the distribution of the lion (6). LGM conditions likely allowed the free movement of the tiger between the Sunda Islands and the continent (7). Post-LGM conditions (d) saw the retreat of the lion range, which, along with more favourable habitat suitability, would have allowed the colonization of the Caspian region via a northern corridor (8), southern corridor (9) or concurrent corridors (8 + 9). Tigers would have been separated by rising sea levels following the LGM through the Strait of Malacca (10), leaving a contiguous population of Continental tigers (11), and Sunda tigers which were likely able to disperse between islands until modern times (e) (12).

rainfall and altitude (Kitchener & Dugmore, 2000) do not reflect the true variability in habitat suitability. Our modelled low habitat suitability on Borneo, compared to Sumatra, Java and Bali, shows that, whilst still forested, the environmental conditions of Borneo are different from those of the other Sunda Islands. Using the 'explain' tool in Maxent (see Appendix S1), it is apparent that precipitation seasonality is the driving factor behind modelled habitat suitability with lower precipitation seasonality causing lower suitability. Borneo exhibits lower prey densities than the other Sunda Islands, associated with seasonal precipitation events that affect flowering and fruiting (Wong *et al.*, 2005), which have been linked to the absence of large carnivores in Borneo (Meijaard, 2004; Wong *et al.*, 2005). Whilst tigers may have been present in Borneo up until recent times (Hooijer, 1963), decreasing habitat suitability from glacial to interglacial conditions, as suggested by Harrison (1996) and supported by our modelling, may have contributed to an extirpation driven by human impacts. It is likely that the inclusion of more complex environmental variables, which incorporate seasonal environmental variability, has highlighted important bioclimatic differences between the Sunda Islands that mirror significant ecological variation.

A key difference between our LGM model and those of previous biogeographical studies is the favourable habitat suitability modelled within the Indian subcontinent, which was predicted as poor habitat by Kitchener & Dugmore (2000). Whilst habitat suitability is reduced in the northern range of the tiger, such as Siberia, it is expanded within the Sunda Shelf and may have led to an increase in overall available tiger habitat during glacial conditions compared to interglacial conditions. Reduced habitat suitability found around the Caspian during the LGM (Fig. 2a) diminishes the likelihood of a viable population during glacial conditions in this region.

The modelling reported here does not support the dispersal of tigers through the Silk Road/Gansu Corridor [Fig. 1(a) – route 3] from China to central Asia and the Caspian (Driscoll *et al.*, 2009). Even considering marginal environments suitable only for dispersal (Fig. 2), this route is deemed improbable. More likely is a colonization of the Amur region from northern China, followed by a post-LGM westward dispersal of tigers through a northern corridor [Figs 1(a) – route 2, 3(8) & 4(d–f)]. Considering the potential rapidity of tiger dispersal – individuals have been known to travel up to 1000 km (Kitchener, 1999), then it is probable that use of the northern corridor and gene flow through optimal interglacial and seasonal conditions would have resulted in low genetic variation between the Amur and Caspian tigers, and this is supported by molecular studies (Driscoll *et al.*, 2009; Wilting *et al.*, 2015).

Significant areas of suitable habitat are modelled along the southern route between the Indian subcontinent and the Caspian during interglacial conditions [Figs 1(a) – route 1, 3(9) & 4(a–c)]. However, a lack of genetic affinity between Caspian and Bengal tigers (Driscoll *et al.*, 2009) argues against this route. Whilst the elevation of the southern route falls within the limits of known tiger dispersal [tigers have been found at altitudes of 4000 m in Bhutan (Sangay & Wangchuk, 2005)], the Hindu Kush mountain range extends directly through the modelled corridor (Fig. 4a–c), and may have acted as a significant barrier to tiger dispersal. A higher tree line in northern Bhutan [4750 m (Miehe *et al.*, 2007)] compared with the central Hindu Kush [3200 m (Schickhoff, 2005)] could account for the presence of tigers at higher altitudes in Bhutan. Alternatively, given the strength of habitat suitability through the southern corridor, colonization of the Caspian may have occurred through simultaneous dispersal via Siberia and the northern corridor and through the southern corridor [Fig. 3(8, 9)].

The Maxent models indicate suitable tiger habitat through the Indian Subcontinent during the LGM, and this is supported by evidence of tigers in Sri Lanka 16.5 ka (Fig. 2a), a time when lower, glacial, sea levels would have allowed colonization from India (Manamendra-Arachchi *et al.*, 2005). Whilst our models appear to show a connection between Korea and Southern Japan during the LGM, which could have allowed the movement of tigers, a 20 km wide Korean/Tsushima Strait existed between the two land masses throughout the LGM (Park *et al.*, 2000) which is 33% further than tigers have been known to swim across seas, even under more benign conditions, and was likely too inhospitable a barrier to allow dispersal. Therefore, it is probable that the extinct Japanese tiger colonized the islands during a previous glacial period, when eustatic sea levels were lower than at the LGM (Rohling *et al.*, 1998), and thus represents a distinctly different population from mainland tigers, as proposed by Kitchener & Dugmore (2000).

The extent of the Toba super-eruption ashfall, in relation to likely suitable tiger habitat at the time, can be seen in Fig. 3(1). Whilst it is probable that many mammal species were able to survive the Toba eruption in geographically

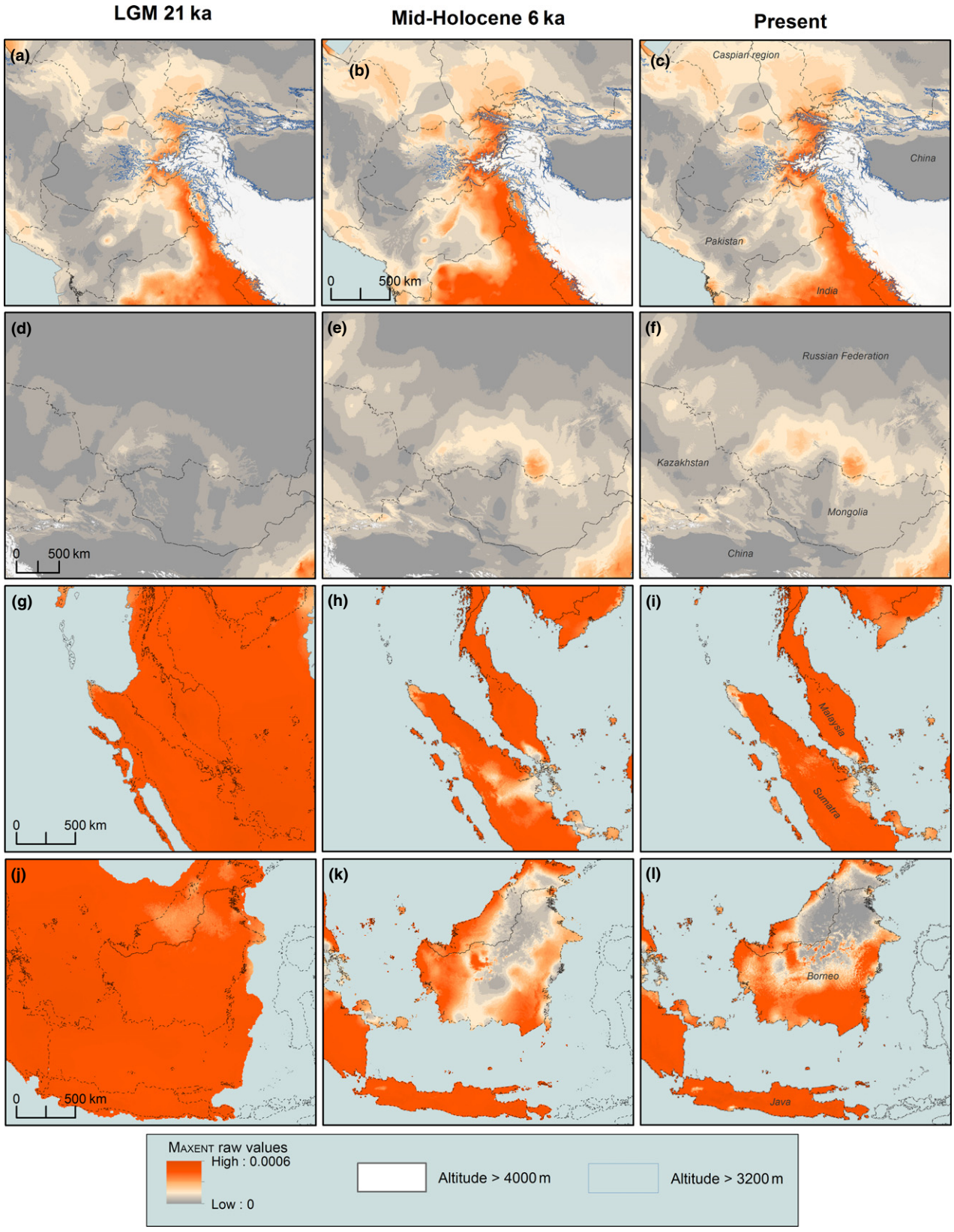

Figure 4 Detailed view of final model projections for the LGM, mid-Holocene and present conditions. In addition to elevations > 4000 m (white areas), included as a possible barrier to tiger dispersal, elevations > 3200 m are shown between India and the Caspian region (a–c) to delineate the regional tree line. Further image tiles show the proposed northern corridor between Amur and Caspian populations [(d–f)], the continental/Sundaland divide (g–i) and range shifts within the Sunda Islands (j–l).

isolated refugia (Prothero, 2004; Louys, 2007), large carnivorans, such as the tiger, are likely to have been particularly susceptible to extinction if confined to limited areas, owing to their requirement for large home range sizes (O'Regan *et al.*, 2002), a notion supported by a demographic reconstruction of the tiger during the Late Pleistocene (Wilting *et al.*, 2015). It is conceivable that direct damage caused by the YTT, and rapid cooling and drought in the following decades, was responsible for the eradication of tigers from most, if not all of, Sumatra and the Malay Peninsula, and might have significantly impacted populations farther afield.

In addition to the direct and indirect environmental effects of the Toba eruption, tigers could have been affected by cooler, stadial conditions lasting around 1000 years after the eruption (Rampino & Self, 1992; Harris, 2008). A subsequent shift from interglacial to glacial conditions (or from present day to LGM tiger distributions by proxy) and its impact on woodlands would have limited the recovery of tigers in the areas affected by Toba [Fig. 3 – ~73ka(+1k)]. An exception could have occurred within the Sunda Islands, where a glacially driven decrease in global sea level during this time (Chappell & Shackleton, 1986) may have allowed the recolonization of Sumatra from Java through temporary land bridges. Even if this opportunity was missed, tigers swim well and have been known to cross rivers 29 km wide and expanses of sea 15 km wide (Kitchener, 1999), so it is conceivable that tigers could have swum between the Sunda Islands even after land bridges were inundated. Indeed, it is likely that tigers swam between Java and Bali into recent times (Kitchener, 1999) a notion supported by the genetic similarity between Javan and Bali tigers, and high morphological affinity between all Sunda Island tigers (Wilting *et al.*, 2015; Fig. 1d).

Southern China/Southeast Asia and the eastern Sunda Islands of Java and Bali represent the 'core' areas of the modelled tiger range that have endured through both glacial and interglacial periods, and they lie outside the major ash fallout zones of the Toba eruption. However, a Sunda Island refugium is unlikely because of the phylogenetic position of the putative South China tiger (*P.t. amoyensis*), which suggests it is ancestral to other tiger lineages (Luo *et al.*, 2004; Driscoll *et al.*, 2009; Wilting *et al.*, 2015), and indicates a population collapse and re-dispersal out of southern China/south-eastern Asia during the Late Pleistocene, including into the Sunda Islands.

At the LGM, and into post-glacial times until the 19th century, suitable habitats existed in India for both the ancestors of modern lions and tigers [Fig. 3(6)], enabling them to be sympatric, but in separate habitats. Indeed, the latest phylogeographical study of lions suggest that they entered the Indian subcontinent around the LGM (Barnett *et al.*, 2014), because of more arid conditions at that time. The disappearance of Eurasian cave lions from northern Asia around 11 ka (Barnett *et al.*, 2009) coincided with climatic and vegetation shifts that favoured the western dispersal of tigers throughout this region, so it is difficult to assess the extent to which competition may have limited one species or the other. The presence of both modern lions and Eurasian cave lions in the Near East may have reinforced the separation between tiger populations of the Indian Subcontinent and the Caspian region.

Our models indicate a recent northern dispersal of tigers, beginning after the LGM, and before the mid-Holocene Climatic Optimum. It is likely that the early rise of civilizations in China, especially along the rivers of the Huanghe catchment over 8 ka (Kong, 1992) resulted in local extirpations of tigers and thus reduced contact, and division between northern continental tigers (Caspian and Amur populations) and southern continental tigers (remaining mainland populations). Major early impacts on Chinese tiger populations are likely because of the danger posed to both humans and livestock, their prized fur and use in traditional medicine. This is supported by studies which show that Amur/Caspian populations are genetically close to Indochinese tigers (Driscoll *et al.*, 2009; Wilting *et al.*, 2015; Fig. 1d), suggesting that there has been insufficient time for any local genetic differentiation despite current isolation of northern populations. However, given their dispersal outside of the core Late Pleistocene habitat of southern Asia, their adaptation to a temperate ecosystem, and their longer term separation compared to more recently fragmented populations of mainland tiger, our results complement the recognition of separate conservation management of northern continental tigers from that of southern continental tigers, as proposed by Wilting *et al.* (2015).

The modelling we present indicates there has been significant separation between continental and Sunda Island populations of tigers since the Last Glacial Maximum, which led to significant population differentiation. This has been proposed by previous genetic, morphological and biogeographical studies (Cracraft *et al.*, 1998; Kitchener, 1999; Kitchener & Dugmore, 2000; Kitchener & Yamaguchi, 2010; Mazák, 2010). There is a clear disparity between current classifications that recognize up to nine tiger subspecies and the contiguity between continental tiger populations during the period of the adaptive radiation of modern populations over the last ca. 100 ka. In comparison with the contiguity of continental tigers, and their proposed post-LGM northward radiation, it is suggested that Asiatic lions constitute the same Evolutionary Significant Unit as North African lions, despite radiating to their currently fragmented position ~21 ka (Barnett *et al.*, 2014). Whilst biogeographical models are not taxonomic tools for deciding the validity of species and subspecies, they provide a deeper time framework against which to judge the significance of genetic and morphological differences between the fragmented populations of today. Thus, they can inform current conservation strategies for endangered widespread species, such as the tiger, and be used to question traditional taxonomies that are based on poor levels of evidence. The scale-dependent nature of taxonomy (Crandall *et al.*, 2000) benefits from an understanding of the changing relationships between populations through space and time. This broader biogeographical approach adds important perspectives to current debates about what we are

trying to conserve both in the wild and captivity, and adds additional scientific weight to arguments for more pragmatic and realistic approaches to conservation.

CONCLUSIONS

Using a niche modelling approach, we have been able to reconstruct the probable dynamic range shifts of the tiger during the Late Pleistocene and Holocene, a period when genetic data suggest that modern populations colonized southern and eastern Asia. We project that throughout this period the tiger's core distribution was in south-eastern Asia, southern China and eastern Sundaland and that tigers colonized north-eastern and central Asia from south-eastern Asia. Owing to continuing geographical contiguity during glacial–interglacial cycles, which allowed for continuing potential gene flow, corroborated by recent molecular studies (Wilting *et al.*, 2015), there is a clear disparity with classifications that recognize six subspecies among mainland populations. The separation of the Sunda Islands from the mainland through sea-level rise since the LGM is consistent with the recognition of potential island population differentiation.

The notion that mainland tiger populations remained contiguous with each other through the Late Pleistocene until Holocene anthropogenic impacts fragmented populations has significant implications for management and conservation. The recognition of only two tiger subspecies (Sunda – *Panthera tigris sondaica,* and Continental tigers – *Panthera tigris tigris*), with the later split into northern and southern populations, which are proposed as separate continental management units (Wilting *et al.*, 2015), would benefit current tiger conservation efforts by allowing more genetic interchange between currently isolated and limited populations. The biogeographical modelling presented here supports only a significant differentiation between Continental and Sundaland tiger populations and thereby offers important insights relevant to both future research and discussions on current tiger conservation.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge support from NERC PhD studentship NE/L002558/1 to D.M.C. and National Science Foundation award 1249313 to A.J.D.

We thank Jane Elith and the anonymous referees for their invaluable comments and suggestions throughout the review process.

REFERENCES

Acharyya, S.K. & Basu, P.K. (1993) Toba ash on the Indian Subcontinent and its implications for correlation of Late Pleistocene Alluvium. *Quaternary Research*, **40**, 10–19.

Barnett, R., Shapiro, B., Barnes, I., Ho, S.Y.W., Burger, J., Yamaguchi, N., Higham, T.F.G., Wheeler, H.T., Rosendahl, W., Sher, A.V., Sotnikova, M., Kuznetsova, T., Baryshnikov, G.F., Martin, L.D., Harington, C.R., Burns, J.A. & Cooper, A. (2009) Phylogeography of lions (*Panthera leo* ssp.) reveals three distinct taxa and a late Pleistocene reduction in genetic diversity. *Molecular Ecology*, **18**, 1668–1677.

Barnett, R., Yamaguchi, N., Shapiro, B., Ho, S.Y.W., Barnes, I., Sabin, R., Werdelin, L., Cuisin, J. & Larson, G. (2014) Revealing the maternal demographic history of *Panthera leo* using ancient DNA and a spatially explicit genealogical analysis. *BMC Evolutionary Biology*, **14**, 70.

Bay, R.A., Ramakrishnan, U. & Hadly, E.A. (2014) A call for tiger management using "reserves" of genetic diversity. *Journal of Heredity*, **105**, 295–302.

Braconnot, P., Otto-Bliesner, B., Harrison, S. *et al.* (2007) Results of PMIP2 coupled simulations of the Mid-Holocene and Last Glacial Maximum – Part 1: experiments and large-scale features. *Climate of the Past*, **3**, 261–277.

Buermann, W., Saatchi, S., Smith, T.B., Zutta, B.R., Chaves, J.A., Milá, B. & Graham, C.H. (2008) Predicting species distributions across the Amazonian and Andean regions using remote sensing data. *Journal of Biogeography*, **35**, 1160–1176.

Chappell, J. & Shackleton, N.J. (1986) Oxygen isotopes and sea level. *Nature*, **324**, 137–140.

Costa, A., Smith, V.C., Macedonio, G. & Matthews, N.E. (2014) The magnitude and impact of the Youngest Toba Tuff super-eruption. *Frontiers in Earth Science*, **2**, 1–8.

Cracraft, J., Feinstein, J., Vaughn, J. & Helm-Bychowski, K. (1998) Sorting out tigers (*Panthera tigris*): mitochondrial sequences, nuclear inserts, systematics, and conservation genetics. *Animal Conservation*, **1**, 139–150.

Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M. & Wayne, R.K. (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution*, **15**, 290–295.

Dinerstein, E., Loucks, C., Heydlauff, A., Wikramanayake, E., Bryja, G., Forrest, J., Ginsberg, J., Klenzendorf, S., Leimgruber, P., O'Brien, T., Sanderson, E., Seidensticker, J. & Songer, M. (2006) *Setting Priorities for the Conservation and Recovery of Wild Tigers: 2005–2015. A User's Guide*. WWF, WCS, Smithsonian, NFWF-STF, Washington D.C., New York.

Driscoll, C.A., Yamaguchi, N., Bar-Gal, G.K., Roca, A.L., Luo, S., Macdonald, D.W. & O'Brien, S.J. (2009) Mitochondrial phylogeography illuminates the origin of the extinct caspian tiger and its relationship to the Amur tiger. *PLoS ONE*, **4**, e4125.

Elith, J., Graham, C.H., Anderson, R.P. *et al.* (2006) Novel methods improve prediction of species 'distributions from occurrence data. *Ecography*, **29**, 129–151.

Garcia-Porta, J., Litvinchuk, S.N., Crochet, P.A., Romano, A., Geniez, P.H., Lo-Valvo, M., Lymberakis, P. & Carranza, S. (2012) Molecular phylogenetics and historical biogeography of the west-palearctic common toads (*Bufo bufo* species complex). *Molecular Phylogenetics and Evolution*, **63**, 113–130.

Geffen, E., Anderson, M.J. & Wayne, R.K. (2004) Climate and habitat barriers to dispersal in the highly mobile grey wolf. *Molecular Ecology*, **13**, 2481–2490.

Goodrich, J., Lynam, A., Miquelle, D., Wibisono, H., Kawanishi, K., Pattanavibool, A., Htun, S., Tempa, T., Karki, J., Jhala, Y. & Karanth, U. (2015) . Available at: www.iucnredlist.org.

Harris, B. (2008) The potential impact of super-volcanic eruptions on the Earth's atmosphere. *Weather*, **63**, 221–225.

Harrison, T. (1996) The palaeoecological context at Niah Cave, Sarawak: evidence from the primate fauna. *Bulletin of the Indo-Pacific Prehistory Association*, **14**, 90–100.

Hernandez, P.A., Graham, C.H., Master, L.L. & Albert, D.L. (2006) The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*, **5**, 773–785.

Herrington, S.J. (1987) Subspecies and the conservation of *Panthera tigris*: preserving Genetic Heterogeneity. *Tigers of the world* (ed.by R.L. Tilson and U.S. Seal), pp. 51–61. Noyes Publications, Park Ridge, NJ.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hooijer, D.A. (1963) Further "Hell" mammals from Niah. *Sarawak Museum Journal*, **11**, 196–200.

Kaplan, J.O., Bigelow, N.H., Prentice, I.C., Harrison, S.P., Bartlein, P.J., Christensen, T.R., Cramer, W., Matveyeva, N.V., McGuire, A.D., Murray, D.F., Razzhivin, V.Y., Smith, B., Walker, D.A., Anderson, P.M., Andrew, A.A., Brubaker, L.B., Edwards, M.E. & Lozhkin, A.V. (2003) Climate change and Arctic ecosystems: 2. Modeling, paleodata-model comparisons, and future projections. *Journal of Geophysical Research*, **108**, 1–17.

Kitchener, A.C. (1999) Tiger distribution, phenotypic variation and conservation issues. *Riding the tiger. Tiger conservation in human-dominated landscapes* (ed.by J. Seidensticker, J. Christie and P. Jackson), pp. 19–39. Cambridge University Press, Cambridge.

Kitchener, A.C. & Dugmore, A.J. (2000) Biogeographical change in the tiger, *Panthera tigris*. *Animal Conservation*, **3**, 113–124.

Kitchener, A.C. & Yamaguchi, N. (2010) What is a tiger? Biogeography, morphology, and taxonomy. *Tigers of the world* (ed.by R. Tilson and P. Nyhus), pp. 53–84. Academic Press, London.

Kong, F. (1992) The destruction of ancient ecological environment and the movement of civilization center in China. *Chinese Geographical Science*, **2**, 382–388.

Kozak, K.H., Graham, C.H. & Wiens, J.J. (2008) Integrating GIS-based environmental data into evolutionary biology. *Trends in Ecology and Evolution*, **23**, 141–148.

Lorenzen, E.D., Nogués-Bravo, D., Orlando, L. *et al.* (2011) Species-specific responses of Late Quaternary megafauna to climate and humans. *Nature*, **479**, 359–364.

Louys, J. (2007) Limited effect of the Quaternary's largest super-eruption (Toba) on land mammals from Southeast Asia. *Quaternary Science Reviews*, **26**, 3108–3117.

Luo, S.-J., Kim, J.-H., Johnson, W.E. *et al.* (2004) Phylogeography and genetic ancestry of tigers (*Panthera tigris*). *PLoS Biology*, **2**, e442.

Luo, S.-J., Zhang, Y., Johnson, W.E., Miao, L., Martelli, P., Antunes, A., Smith, J.L.D. & O'Brien, S.J. (2014) Sympatric Asian felid phylogeography reveals a major Indochinese-Sundaic divergence. *Molecular Ecology*, **23**, 2072–2092.

Manamendra-Arachchi, K., Pethiyagoda, R., Dissanayake, R. & Meegaskumbura, M. (2005) A second extinct big cat from the late quaternary of Sri Lanka. *The Raffles Bulletin of Zoology*, **12**, 423–434.

Martínez-Meyer, E., Peterson, A.T. & Hargrove, W.W. (2004) Ecological niches as stable distributional constraints on mammal species, with implications for Pleistocene extinctions and climate change projections for biodiversity. *Global Ecology and Biogeography*, **13**, 305–314.

Mazák, V.J. (1996) *Der tiger*. Westarp Wissenschaften, Magdeburg.

Mazák, J.H. (2010) Craniometric variation in the tiger (*Panthera tigris*): implications for patterns of diversity, taxonomy and conservation. *Mammalian Biology - Zeitschrift für Säugetierkunde*, **75**, 45–68.

Mazák, V.J., Groves, C.P. & van Bree, P.J. (1978) On a skin and skull of the Bali tiger, and a list of preserved specimens of *Panthera tigris* balica (Schwarz, 1912). *Zeitschrift für Säugetierkunde*, **43**, 108–113.

Mazák, J.H., Christiansen, P. & Kitchener, A.C. (2011) Oldest known pantherine skull and evolution of the tiger. *PLoS ONE*, **6**, 1–11.

Meijaard, E. (2004) Biogeographic history of the Javan leopard *Panthera pardus* based on a craniometric analysis. *Journal of Mammalogy*, **85**, 302–310.

Miehe, G., Miehe, S., Vogel, J., Co, S. & La, D. (2007) Highest treeline in the northern hemisphere found in southern Tibet. *Mountain Research and Development*, **27**, 169–173.

Mondol, S., Bruford, M.W. & Ramakrishnan, U. (2013) Demographic loss, genetic structure and the conservation implications for Indian tigers. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 1–10.

Muscarella, R., Galante, P.J., Soley-Guardia, M., Boria, R.A., Kass, J.M., Uriarte, M. & Anderson, R.P. (2014) ENMeval: an R package for conducting spatially independent evaluations and estimating optimal model complexity for Maxent ecological niche models. *Methods in Ecology and Evolution*, **5**, 1198–1205.

Nowell, K. & Jackson, P. (1996) *The wildcats: a status survey and conservation action plan*. IUCN, Gland, Switzerland.

Oppenheimer, C. (2002) Limited global change due to the largest known Quaternary eruption, Toba ~ 74 kyr BP? *Quaternary Science Reviews*, **21**, 1593–1609.

O'Regan, H.J., Turner, A. & Wilkinson, D.M. (2002) European quaternary refugia: a factor in large carnivore extinction? *Journal of Quaternary Science*, **17**, 789–795.

Park, S.-C., Yoo, D.-G., Lee, C.-W. & Lee, E.-I. (2000) Last glacial sea-level changes and paleogeography of the Korea (Tsushima) Strait. *Geo-Marine Letters*, **20**, 64–71.

Pearson, R.G., Raxworthy, C.J., Nakamura, M. & Townsend Peterson, A. (2006) Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography*, **34**, 102–117.

Phillips, S.J. & Dudík, M. (2008) Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, **31**, 161–175.

Phillips, S.J., Anderson, R.P. & Schapire, R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.

Prothero, D.R. (2004) Did impacts, volcanic eruptions, or climate change affect mammalian evolution? *Palaeogeography, Palaeoclimatology, Palaeoecology*, **214**, 283–294.

R Core Team (2015) *R: a language and environment for statistical computing*. Vienna, Austria. URL https://www.R-project.org/.

Rampino, M.R. & Self, S. (1992) Volcanic winter and accelerated glaciation following the Toba super-eruption. *Nature*, **359**, 50–52.

Renner, I.W., Elith, J., Baddeley, A., Fithian, W., Hastie, T., Phillips, S.J., Popovic, G. & Warton, D.I. (2015) Point process models for presence-only analysis. *Methods in Ecology and Evolution*, **6**, 366–379.

Robock, A., Ammann, C.M., Oman, L., Shindell, D., Levis, S. & Georgiy, S. (2009) Did the Toba volcanic eruption of ~74 ka B.P. produce widespread glaciation? *Journal of Geophysical Research*, **114**, 1–9.

Rohling, E.J., Fenton, M., Jorissen, F.J., Bertrand, P., Ganssen, G. & Caulet, J.P. (1998) Magnitudes of sea-level lowstands of the past 500,000 years. *Nature*, **394**, 162–165.

Rose, W.I. & Chesner, C.A. (1987) Dispersal of Ash in the Great Toba Eruption, 75 ka. *Geology*, **15**, 913–917.

Sangay, T. & Wangchuk, T. (2005) Tiger Action Plan for the Kingdom of Bhutan 2006–2015.

Schickhoff, U. (2005) The upper timberline in the Himalayas, Hindu Kush and Karakorum: a review of geographical and ecological aspects. *Mountain ecosystems. Studies in treeline ecology* (ed.by G. Broll and B. Keplin), pp. 275–354. Springer, Berlin.

Shane, P., Westgate, J., Williams, M. & Korisettar, R. (1995) New geochemical evidence for the youngest Toba Tuff in India. *Quaternary Research*, **44**, 200–204.

Sunquist, M.E. (1981) The social organization of tigers (*Panthera tigris*) in Royal Chitawan National Park, Nepal. *Smithsonian Contributions to Zoology*, **336**, 1–98.

Tao, W., Huijun, W. & Dabang, J. (2010) Mid-Holocene East Asian summer climate as simulated by the PMIP2 models. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **288**, 93–102.

Taylor, K.E., Stouffer, R.J. & Meehl, G.A. (2012) An overview of CMIP5 and the experiment design. *Bulletin of the American Meteorological Society*, **93**, 485–498.

Varela, S., Lobo, J.M., Rodríguez, J. & Batra, P. (2010) Were the Late Pleistocene climatic changes responsible for the disappearance of the European spotted hyena populations? Hindcasting a species geographic distribution across time. *Quaternary Science Reviews*, **29**, 2027–2035.

Veloz, S.D. (2009) Spatially autocorrelated sampling falsely inflates measures of accuracy for presence-only niche models. *Journal of Biogeography*, **36**, 2290–2299.

Walston, J., Robinson, J.G., Bennett, E.L. *et al.* (2010) Bringing the tiger back from the brink-the six percent solution. *PLoS Biology*, **8**, 6–9.

Waltari, E., Hijmans, R.J., Peterson, A.T., Nyári, A.S., Perkins, S.L. & Guralnick, R.P. (2007) Locating Pleistocene refugia: comparing phylogeographic and ecological niche model predictions. *PLoS ONE*, **2**, 1–11.

Whitmore, T.C. (1984) *Tropical rain forests of the Far East*. Oxford University Press, Oxford.

Williams, M.A.J., Ambrose, S.H., van der Kaars, S., Ruehlemann, C., Chattopadhyaya, U., Pal, J. & Chauhan, P.R. (2009) Environmental impact of the 73 ka Toba supereruption in South Asia. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **284**, 295–314.

Wilting, A., Christiansen, P., Kitchener, A.C., Kemp, Y.J.M., Ambu, L. & Fickel, J. (2011) Geographical variation in and evolutionary history of the Sunda clouded leopard (Neofelis diardi) (Mammalia: Carnivora: Felidae) with the description of a new subspecies from Borneo. *Molecular Phylogenetics and Evolution*, **58**, 317–328.

Wilting, A., Sollmann, R., Meijaard, E., Helgen, K.M. & Fickel, J. (2012) Mentawai's endemic, relictual fauna: is it evidence for Pleistocene extinctions on Sumatra? *Journal of Biogeography*, **39**, 1608–1620.

Wilting, A., Courtiol, A., Christiansen, P., Niedballa, J., Scharf, A.K., Orlando, L., Balkenhol, N., Hofer, H., Kramer-Schadt, S., Fickel, J. & Kitchener, A.C. (2015) Planning tiger recovery: understanding intraspecific variation for effective conservation. *Science Advances*, **1**, 1–13.

Wong, S.Te., Servheen, C., Ambu, L. & Norhayati, A. (2005) Impacts of fruit production cycles on Malayan sun bears and bearded pigs in lowland tropical forest of Sabah, Malaysian Borneo. *Journal of Tropical Ecology*, **21**, 627.

Xue, H.R., Yamaguchi, N., Driscoll, C.A., Han, Y., Bar-Gal, G.K., Zhuang, Y., Mazak, J.H., Macdonald, D.W., O'Brien, S.J. & Luo, S.J. (2015) Genetic ancestry of the extinct Javan and Bali Tigers. *Journal of Heredity*, **106**, 1–11.

Yamaguchi, N., Cooper, A., Werdelin, L. & Macdonald, D.W. (2004) Evolution of the mane and group-living in the lion (*Panthera leo*): a review. *Journal of Zoology*, **263**, 329–342.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Ecological niche model evaluation, parameter selection and projection process to past conditions.

BIOSKETCH

David M. Cooper is a PhD student and biogeographer whose research focuses on the evolutionary history of big cats over the Late Pleistocene and Holocene. Thus, far ecological niche modelling onto past climatic conditions has been a key theme.

D.M.C., A.J.D., A.C.K. and B.M.G. are members of the Vertebrate Biogeography Research Group at the National Museums of Scotland and University of Edinburgh. This group brings together research in taxonomy, morphometrics and phylogeography in the Department of Natural Sciences at the National Museums of Scotland, with physical geography, ecology, GIS and modelling in the School of GeoScience at the University of Edinburgh.

Author contributions: A.C.K., A.J.D. and D.M.C. designed the research; A.W., A.K.S. and D.M.C. collated the data; D.M.C, A.C.K., A.J.D. and B.M.G. performed the research; all co-authors contributed to writing that was led by D.M.C.

Editor: Jane Elith